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DESTABILIZATION OF THE MILK FAT EMULSION OF ICE CREAM

by

THABIT ALSAFAR

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies for acceptance, a thesis entitled

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submitted by Thabit Alsafar in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

TO MY WIFE

In appreciation of her invaluable
help and encouragement.

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ABSTRACT

The destabilized fat content of commercial "soft-serve" and packaged hardened ice creams were found to range from 18 to 100% and 8 to 11% respectively. These results confirm the findings of other investigators regarding the seriousness of this defect in ice cream especially in the "soft-serve" segment of the ice cream industry. Contrary to published information, the ice cream mixes examined, both commercial and experimental, contained considerable quantities of destabilized fat ranging from 4 to 9%.

Results obtained from experimental mixes and corresponding ice cream revealed that the destabilization of butterfat was proportional to the butterfat content. The greatest increase in destabilization occurred during the normal freezing operation (approximately 10 minutes). Substantial additional destabilization continued during prolonged agitation in the freezer for a period of 15 to 30 minutes, when the temperature of the ice cream was maintained at 23°F.

Destabilization of the butterfat in experimental ice cream was influenced by the type of emulsifier incorporated, but no effect was observed on mixes.

Liquid type emulsifiers, which are hydrophilic, such as Tween 80, caused the greatest destabilization. In these emulsifiers the improper balance between hydrophilic and lipophilic properties apparently affect adversely the stability of the butterfat emulsion. Increases in the concentration of emulsifiers caused greater destabilization. Over-emulsification, as a consequence of the concentration of unsaturated fatty acids and the presence of the polyglycol chain in the molecular structure of these emulsifiers is a possible cause.

The addition of sodium oxalate, sodium hexametaphosphate and sodium tetraphosphate at two concentrations, 0.1 and 0.2%, was effective in reducing the destabilized fat content of ice cream. This was especially true of sodium tetraphosphate. All the [illegible] salts were more effective at the highest concentration added. The role of these salts in stabilizing the butterfat emulsion is discussed.

The ice cream made from butter oil as a source of fat contained a greater quantity of destabilized butterfat than ice cream made from cream. The difference, however, was much less than anticipated. This indicates that a product with normal texture characteristics

could be produced under the severe freezing conditions prevailing during the manufacture of "soft-serve" ice cream, especially if full advantage is taken of the stabilizing effect of certain emulsifiers and stabilizing salts demonstrated in this study.

Changes in the structure of ice cream and the appearance of butterfat globules, destabilized butterfat and membrane material were observed by microscopical techniques, including polarized light and electron microscope examinations.

A modification of the dilution and separation method of Schulz <u>et al</u>. (1959) for measuring the destabilization of the butterfat emulsion proved to be satisfactory in this investigation.

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DESTABILIZATION OF THE MILK FAT EMULSION OF ICE CREAM

INTRODUCTION

Normally, a milk fat emulsion is stabilized by an adsorbed layer or membrane around the fat globule which is relatively stable to any agitation received in processing, and consequently, the emulsion remains relatively unchanged in market milk, cream, evaporated milk, and ice cream mix. In the processing of other milk products, especially butter and butter oil, the stability of the milk fat globule is weakened and finally destroyed in order to release the enclosed butterfat which acts as a cementing material which unites the remaining globules into granules. In the production of butter oil, the application of heat after churning brings about aggregation, to complete the destabilization process, particularly when other conditions are favorable. Chemical destabilization with acids, alkalis and surface-active agents is the basis for most of the tests developed for butterfat, and also for the production of butter oil directly from cream by eliminating the agglomeration or churning procedure.

Extensive destabilization of the milk fat emulsion causes serious defects in ice cream. This is especially true in the manufacture of soft ice cream for immediate sale without subsequent hardening, as in this operation the product is frequently subjected to severe agitation, especially during freezing. In hardened ice cream which is used in the manufacture of novelties, such as sandwiches and ice cream bars, a certain additional degree of stiffness and dryness is desirable. Apparently, this quality is contributed to by a limited destabilization of the milk fat emulsion. Recently, investigations have shown that these attributes are favored, at least primarily, by an agglomeration of fat globules which is facilitated by the incorporation of certain emulsifiers. Ice cream containing some agglomerated fat exhibits a slow melt down, presumably caused by the physical structure of the milk fat aggregates. When the agglomeration which preceeds destabilization,is carried beyond a certain point, as commonly occurs in the freezing of soft-serve ice cream, churning of the milk fat occurs with the undesirable formation of visible hard granules of butter.

It is apparent from the foregoing that destabilization of the milk fat emulsion, with the accompanying clumping and churning of the milk fat globules into granules or

aggregates, may be contributed to by the mix composition, (milk fat sources, emulsifiers and salt content), as well as faulty processing (homogenization and freezing).

In present day mechanization of ice cream making the body and texture requirements are very exact. In the manufacture of packaged novelties, ice cream is required to have body characteristics which will permit the use of packaging equipment without damaging the quality of the product. In soft-serve ice cream, the requirements are somewhat similar in that the product is required to resist melting. The object of this research is to investigate the effect of certain factors such as fat content, type of fat and emulsifier,concentrations of emulsifiers and added salts on the stability of the fat emulsion in ice cream. Procedures have been developed which, it is hoped, will be used in determining the degree of destabilization of milk fat emulsion in ice cream quantitatively and qualitatively.

REVIEW OF LITERATURE

The Milk Fat Globule: Theories of Formation and the Characteristics.

The milk fat globule is a complicated physico-chemical structure consisting of mixed triglycerides protected by a surface layer of phospholipid material, lipoprotein and other constituents. The surface layer is referred to as the fat globule membrane, and the behavior of the globule under chemical and physical treatments is attributed to the changes which take place in the membrane material. Knowledge regarding the mechanism of the formation of the milk fat globule in the secretory cells of the mammary gland and the chemical and physical structure of the surface layer is incomplete. Rimpila and Palmer (1935) concluded that the milk fat globule may be secreted and covered by the membrane material before the milk plasma is completely formed. According to Mulder (1957) the milk fat globules and the milk plasma are manufactured by the same lacteal glands but at different sites. He stated that the fat globule is probably surrounded by a thin "protoplasm-like" membrane before reaching the milk plasma. Hansson (1949) investigated the milk fat globules with the aid of the electron microscope. He concluded that the globules are elaborated simultaneously

with the milk plasma and also that they are oval in shape. The oval shape of the globules observed by Hansson was questioned by King (1955a) who stated that the preparation of the specimen for the electron microscope studies might cause this oval-shaped appearance.

According to Schwarz (1947), the fat globule of 35% fat unpasteurized cream shows an elongated form surrounded by three layers: an inner thin, dark layer consisting of protein-like material; a layer made up of a series of small beads, considered to be phospholipids, and a layer largely diffuse of a slimy protein-like substance carrying the scattered enzymes. Jack and Dahle (1937), presented some evidence supporting the probability of the existence of a double layer membrane on the surface of the fat globules, the outer layer of which must be removed in order to secure centrifuged cream containing more than 65% fat. This was based on the observation conducted by these authors concerning the electrophoretic mobilities and the lipoid phosphorus content. They found that the electrophoretic mobilities and the lipoid phosphorus content were constant in cream containing 17 to 65% fat, however, the electrophoretic mobilities were increased with an increase in fat content above 65% and the lipoid phosphorous content was decreased.

They concluded that above 65% fat, some tightly held phospholipid material in the fat globule membrane, must be removed in order to secure cream of more than 65% fat. This was supported by Palmer (1944). Hare et al. (1952) claimed that the protein portion of the membrane material does not resemble any of the other protein found in milk. This was supported by Mulder and Manger (1958). However, Brunner et al. (1953b) indicated that it may be a protein of globulin-like nature or even lactoglobulin.

A washing technique has been used for many years for the isolation of the membrane material from the milk fat globule for chemical and physical analysis. The first washing technique was developed by Lehmann and applied by Voltz in 1904 according to King (1955a). The method of repeated dilution and separation was employed by Palmer and his associates in their studies on the nature of the fat globule membrane during the period from 1924 to 1945. Their method was based on churning the washed cream and recovering the membrane material from the butter milk and butter plasma. Palmer (1944) concluded that the protein material was the main component of the membrane substance. Palmer and Weise (1933) examined a sample of milk containing 3.5% fat and reported 0.023 - 0.031% crude membrane material which corresponds to 0.66 - 0.89 g of membrane

material per 100 g of fat. Jenness and Palmer (1945b) found 0.71 - 1.20 g of membrane substance per 100 g of milk fat. Palmer and Weise (1933) identified the components of the phospholipid membrane material as lecithin, cephalin and sphingomylin. Palmer (1944) and Jenness and Palmer (1945a) reported that the phospholipid membrane material is more surface-active than the protein portion and the specific emulsion-stabilizing agent of the milk fat globule is a protein phospholipid complex lipoprotein. According to Mulder et al. (1953), the typical flavor of buttermilk may be attributed to the constituents of the phospholipid material, especially the lecithin. The fat globule membrane also contains high-melting triglycerides which were first reported by Palmer and Weise (1933).

The origin and nature of the high-melting triglycerides were further investigated by Jenness and Palmer (1945a). These authors found the high-melting triglycerides concentrated in the butter plasma and buttermilk. The occurrence of the high-melting triglycerides in buttermilk was explained by the presence of certain amounts of unchurned fat globules or of butter granules, It was believed that the butter plasma contained more of the high-melting triglycerides because of the affinity

between the molecules of the high-melting triglycerides and the fatty acid chain of the phospholipid material.

The bound water content of the milk fat globule was investigated by Pyenson and Dahle (1938). They stated that the water-binding capacity of the membrane material and casein were similar, and the presence of large portions of the bound water in cream is attributed to the hydrophilic properties of the fat globule membrane. They also reported that on heating, the bound water content of the membrane material showed a marked decrease.

Milk fat globules possess electrophoretic properties which permit them to move in an electric field. The charges are negative at a high pH and positive at a low pH. According to King (1955a), Moyer (1940) studied the isoelectric points of the washed and unwashed fat globules. He reported that the isoelectric point of the unwashed fat globules was pH 4.5, while that of the washed fat globules was pH 3.7 - 3.8.

The physical arrangement of the constituents of the fat globule membrane is not clearly understood. However, the chemical composition of the constituents, the nature of orientation of the constituents and the distance between them undoubtedly play an important role in their

natural arrangement. Rimpila and Palmer (1935) were the first to propose the general concept of an orientation of the membrane material of the milk fat globule. Bird et al.(1937) postulated that the protective membrane material does not form a continuous membrane at the globule surface, instead it is held by a force which is randomly distributed around the surface of the fat globule. The protective material is stated to be of two types: 1) a phospholipid-protein complex oriented at the fat side of the interface, and 2) surface-active components from milk plasma situated in the water side of the interface. Of these, casein is probably the most important. Jenness and Palmer (1945b) demonstrated that the strength of the bond between lipoprotein and the high-melting triglycerides, may be due to Van der Waal's forces, Moore (1955). This was based on the ability of the lipoprotein material to draw a considerable proportion of the high-melting triglycerides into the butter plasma when butter is melted. King (1955a) suggested the following arrangement for the fat globule membrane components. The primary layer consisting of a monolayer of orientated phospholipid molecules, whose lipophilic chains adhere to the fat phase, and the hydrophilic group facing the aqueous phase. The primary layer is followed by a secondary layer composed of uncoiled

molecules of the membrane protein, with the main chain facing the surface of the fat globule. The occurrence of the phospholipid material in the membrane of the fat globule was further investigated by Morton (1954). He stated that the fat globule in milk is surrounded by a continuous protein membrane to which small submicroscopical lipoprotein particles are adsorbed. He supported his finding by electron microscope photomicrographs.

Factors Associated with the Stability of the Milk Fat Globule.

The stability of the fat emulsion of milk and milk products is of great practical and theoretical significance. In some milk products, it is desirable to maintain as far as possible the original fine dispersion of the fat globules, while in others, particularly ice cream, the dispersion is improved by homogenization. The extent to which fat globules may retain their original properties is closely related to the behavior of the surface layers surrounding them. Among the factors governing the behavior of the fat globules during processing are: the chemical and physical state and structure of the milk fat, the mechanical and electrical properties and the influence of other milk constituents. The mechanism by which these factors influence the behavior of milk fat globules is

complex and far from being completely understood. It has been demonstrated that the attraction between fat globules can be increased by increasing the hydrophobic property of their surface layer. This can be achieved by an alteration of the membrane material either partially, or completely, or by depositing on the fat membrane a hydrophobic coating consisting of milk fat, King (1955a). King further explained that clumping takes place when the fat globules are in a partially solidified state containing the crystalline and liquid fraction of the fat in an optimum ratio. For clumping to take place, a certain amount of liquid fat must be released from some fat globules. The release of liquid fat from fat globules may be achieved by treatments causing damage to the surface layer of the milk fat globule. Some of the released liquid fat spreads over the surface of the fat globules and when two or more fat globules approach each other the liquid fat fuses them together by the force of cohesion. The individual fat globules of clumps or aggregates may still be visible under the polarizing microscope. The temperature at which clumping takes place was also discussed by King. At low temperature, when only a small portion of the fat inside the fat globules is present in the liquid state no clumping occurs. However, at a temperature close

to or above the melting point of the fat, the crystalline structure in the globules disappears and the clumps form large fat globules; this stage is termed 'coalescence'.

King (1955a) classified the factors which may cause destabilization of the milk emulsion as: 1) mechanical, such as agitation and homogenization; 2) thermal factors such as freezing, heating and temperature treatments; 3) factors of a physico-chemical nature such as washing, drying and action of reagents and micro-organisms.

The Influence of Agitation.

The effect of agitation on the stability of the milk fat emulsion is frequently observed during transportation, churning, homogenization and extended agitation of ice cream mixes in the freezer. The effect of agitation on the behavior of the fat globules and the formation of butter have been discussed by King (1951, 1952, 1953). According to King (1953), during agitation, the milk fat globules undergo two kinds of physico-chemical change. The fat globules may aggregate to form large units, initiating the first stage in butter granule formation or the smaller globules, with a diameter of 2 - 4 microns, may be broken up and dispersed as very small globules with a diameter of 0.5 - 1.0 microns. Which type of physico-

chemical change takes place depends on the size of the fat globules, the temperature and extent of the treatment. Dolby (1957) pointed out that the number of small fat globules to be formed as a result of agitation depends on the pH of the milk or cream. The small fat globules predominate when the pH of the agitated milk or cream is 7.30 - 7.45. This indicates that high acidity encourages clumping or formation of large fat globules. The fundamental aspects of the continuous butter making was explained by King (1952) who stated that agitation incorporates air in the cream plasma causing some of the fat globules to spread part of their membrane with some liquid fat upon contact with air bubbles. The fat globules which are in contact with the air cells retain some liquid fat on their surfaces which permits them to aggregate if conditions are favorable. King (1953) stated that the behavior of the globule surfaces represents the central phenomena of churning and some of the fat globules survive the harsh treatment during churning because of the crystalline structure of the fat inside the fat globule. It was King (1950b) who reported that about 20 - 50% of the total fat of butter is present in the form of globules, while the remaining fat consists of "free fat", a mixture of liquid and crystalline fat.

Holland and Herrington (1953) studied the effect of temperatures on the churning time of milk fat in cream containing 40% fat. These authors reported that there is an optimum relationship between the liquid fat and solid fat for churning to take place. If either is increased, the churning time is lengthened. Churning is not normal when the fat is completely solidified or liquid.

King (1955a) elaborated on the changes occurring during the whipping of cream. A three-phase system is formed. The air bubbles are incorporated in the plasma which contains the fat globules in a clumped state. Only incipient churning normally takes place because most of the fat is in the solid state. King also stated that in one continuous butter making process (Creamery Package), the high fat content cream is subjected to homogenization while warm, which causes a complete breakage of the fat-in-water emulsion. In the Cherry-Burrell process (Gold'n Flow), the fat globules are agitated before the second separation in order to obtain cream of high fat content. deMan and Wood (1958) found that destabilization of butter fat was more complete when the duration of the mechanical agitation was increased.

Agitation of raw milk, when the fat is mostly liquid,

greatly activates the lipase naturally present in milk. This activation effect was attributed to the alteration in the arrangement of the surface layer of the fat globules, Krukovsky and Sharp (1940). This finding was supported by Dunkly and Smith (1951).

According to Brunner et al. (1953b), homogenization causes an alteration of the arrangement of the membrane material of the fat globules. In homogenized milk, the major plasma proteins are to a certain extent mixed with the protein portion of the membrane substance. This finding was supported by Trout (1953).

Loo et al. (1950) claimed that cavitation might be involved in fat clumping and protein denaturation of homogenized milk or cream. Tarassuk and Richardson (1941) stated that agitation, homogenization and temperature treatment of milk or cream may cause disruption or distortion of the membrane material of the fat globules. Dahle and Bradly (1932) investigated the effect of whipping time on texture and size of ice crystals in ice cream. They concluded that as freezing and whipping time increased from 5 to 15 minutes, at the same temperature, the average size of ice crystals increased from 0.072 to 0.116 mm at 24.5° F. A superior textured ice cream was obtained with shorter whipping and freezing time. This was accounted

for by the conversion of more water into ice crystals in the freezer where conditions are ideal for the formation of fine ice crystals. Sommer (1946) stated that homogenization of ice cream mixes decidedly smooths the texture of the product. This, he accounted for by the finer dispersion of the fat globules, which presents mechanical obstructions to ice crystal growth and favors smaller air cell formation. This observation was confirmed by Cremers (1954). Arbuckle (1940) investigated the influence of acidity on the texture and structure of ice cream. He concluded that ice crystals are larger in ice cream with high acidity, and that smaller ice crystals are formed when the pH of the mix is nearer neutrality. Sommer (1951) stated that during agitation, some of the air cells escape, depending on the strength of the thin film formed on the surface of the air cells, (lamella), which is weakened by continuous whipping. Knightly (1959) reported that low fat content ice cream mixes resist churning more than high fat content mixes. This was explained by the fact that in low fat ice creams there are fewer globules and consequently, there is less chance for agglomeration.

According to Frazuer (1959b) the churning of butter-fat is the most serious problem in the soft-serve ice

cream operation. He further explained that the softer the fat, because of the presence of more unsaturated fatty acids, the quicker the churning. Piper (1959) pointed out that hard, lumpy particles of butter are common in the soft-serve freezer, especially where high fat content mixes are used.

Sommer (1951) claimed that the properties of the surface of milk fat globules are the consequence of purely physical factors. The re-emulsification is a result of two opposing behaviors: one is the disruption of the disperse phase into small particles, and the other is the collection of those particles, or aggregation by agglomeration or coalescence. In order to avoid the second case, a quick adsorption and re-arrangement must take place. Josephson and Dahle (1936) explained the re-establishment of a lipoprotein membrane on the newly formed fat globules in ice cream mixes, when butter or butter oil are used as the fat source. This is essential for normal whipping quality during the freezing process. When butter is emulsified in skimmilk, ordinary buttermilk, or washed cream buttermilk, very stable emulsions were obtained. However, only when washed cream buttermilk is used, a mix with normal whipping property was obtained. On the other hand, when

butter and butter oil were emulsified with phospholipids from milk or eggs, only emulsified butter produced stable emulsion of normal whipping properties. This was attributed to the presence of curd in butter, which combines with phospholipids to form the lipoprotein complex.

According to Kloser and Keeney (1958), the degree of fat destabilization during freezing increased as the fat content of the mix was increased. Very little fat destabilization occurred in the 4% ice milk products, whereas approximately 44% of the fat in the 14% mix had been destabilized after 30 minutes in the freezer. Their results revealed that the monoglycerides which contained the lower molecular weight saturated fatty acids, capric and lauric, proved to be powerful destabilizers. The monoglycerides which contained high molecular weight saturated fatty acids, such as palmetic and stearic, were not strong fat destabilizing agents. It was also found that monoglycerides containing unsaturated high molecular weight fatty acids, such as oleic acid, were effective destabilizing agents. Certain surface-active agents were tried by these investigators who reported that Spans (Sorbitan

derivative), were not good drying agents, and do not promote extensive fat destabilization. On the other hand, Tweens, (polyoxyethylene derivatives), showed excellent drying properties and caused extensive fat destabilization in ice cream. The emulsifiers which contained unsaturated or short chain fatty acids proved to be the most effective drying agents and caused the most fat destabilization. Thus, Tween 80, an oleic acid derivative, caused the highest fat destabilization recorded in the experiments of these investigators. The effect of Tween 80 emulsifier on the dispersion properties of whole milk powder was studied by Mather and Hollender (1955). These authors found that Tween 80 emulsifier improved the dispersion property of whole milk powder, but the product churned when it was reconstituted with mechanical agitation. Bassett (1958) also reviewed different types of emulsifiers used in ice cream.

Keeney and Josephson (1958) observed that the longer the mix was agitated in the freezer, the greater was the time required for the samples to melt freely. This was true for both the control and ice creams containing emulsifiers. However, on melting, the control ice cream left a transparent film on the side of the container similar to skimmilk foam. The ice cream with emulsifier

which received minimum agitation left a similar film, while those samples which were whipped extensively showed an appearance similar to that of whipped cream, a product which depends on partial churning for foam stability. The effect of emulsifiers on the dryness of ice cream was assumed to be related to the controlling action of the emulsifiers on the conditions favoring a stable fat dispersion. Dryness is dependent on the aggregation of fat particles and the process of producing dry "melt-resistant" frozen ice cream is similar to the production of whipped cream. Another concept advanced was that the emulsifiers are adsorbed on the air-serum interface which strengthens the film, thus making possible the fine, stable air cell structure. They concluded that dryness is dependent on the aggregation of fat particles.

The effect of added salts on the stability of the fat emulsion in ice cream was investigated by Hening and Dahlberg (1929), Doan (1930), Keith et al. (1935), Dahle and River (1940, Frazuer (1958a), Knightly (1959), and Keeney (1962). All these authors agreed that certain salts such as the citrates and phosphates added to the stability of the fat emulsion, while calcium and magnesium decreased the stability of the emulsion. The explanations for the action of the salts were all

based on speculations.

The Influence of Freezing.

The effect of freezing on the stability of the butter fat emulsion has received much investigation. Dahle (1941) gave a comprehensive report on frozen cream. He concluded that the membrane loses its healing property when the milk or cream is frozen. This was attributed to the following:

1. Pressure caused by large ice crystal formation;
2. Separation of the fat globules from the plasma by the frozen portion;
3. Increase in dehydration of the membrane of the fat globules.

Trout and Sheid (1943)found more destabilization of fat when the cream was frozen slowly. According to Bell and Sanders (1946), the quality of the raw material , and the treatment it receives before freezing greatly effects the stability of the fat emulsion. They showed that cream from milk which was not cooled before being separated at 37°C was more stable on freezing than cream from milk which was cooled before separation at 37°C. Doan and Baldwin (1936) reported that if the center of a can of cream is frozen before the surface, a pressure of expansion is developed, and this will be resisted by

the pressure from the surface after it is frozen. The force between the two pressures will injure the fat globules.

Trout and Sheid (1943) investigated the effect of pasteurization on the fat stability in products to be frozen. They concluded that cream which was pasteurized at 160°F for 15 minutes showed less destabilization upon freezing than cream which was pasteurized at 150°F for 30 minutes, or at 185°F for 5 minutes. Webb and Hall (1935) pointed out that homogenization showed more effect in preventing fat destabilization of frozen 10 - 20% cream than with 30 - 40% cream. They also stated that cream from Jersey milk showed more destabilization upon freezing than cream from Holstein milk. This was accounted for by the fact that large fat globules resist destabilization less than small fat globules, an observation which was supported by Bell and Sanders (1945). Polonovski and Neuzil (1949) suggested that freezing draws some of the bound water away from the membrane of the fat globule, and causes an alteration of the membrane material. According to this investigator, cream which was frozen soon after separation showed less destabilized fat on freezing than cream which was held 4 hours after separation and before freezing. Necherson <u>et al</u>. (1960) stated that frozen homogenized milk showed

less fat destabilization than frozen non-homogenized milk. Lagoni and Peters (1961), attributed the effect of freezing on the stability of the fat emulsion in frozen cream to the difference in the heat conductivity between water and fat. This difference can be minimized by quick freezing and thawing. Webb and Hall (1935) recommended the use of sucrose or milk-solid-not-fat added to cream before freezing to reduce fat destabilization. They also reported that the higher the fat content in the cream the higher the destabilization of butterfat caused by freezing.

<u>The Influence of Chemical and Biological Factors</u>.

Certain organic and inorganic compounds attack the membrane of the fat globules when added to milk or milk products. The chemicals used in different methods of testing milk and milk products for fat are familiar examples.

Patton (1952) and Stine and Patton (1951, 1952, 1953) used chemical reagents to produce butter oil. Patton (1952) stated that the effectiveness of the organic compounds in breaking the fat emulsion depends on their ability to penetrate and disperse the proteinaceous material of the fat globule membrane. Stine and Patton (1952) stated that certain surface-active agents can

break the milkfat emulsion when added to cream at a level of 10% or less. King (1957) pointed out that certain surface-active agents added to the surface of whole milk may either bring to the surface separate or clumped fat globules or patches of destabilized fat; alternately, they may be caused to sink. Alcohols, in general, can bring the fat globules to the surface of the milk much faster than mechanical disturbances, while glycerols are less active. Spans,(sorbitans), and Tweens,(polyoxyethylenes), effect a sweeping or removal of the fat globules from the surface of the milk. The action of the surface-active agents was accounted for by the fact that they are adsorbed on the surface of the fat globules and at the same time they spread out on the surface of the freshly formed fat droplets sweeping their surface clean of fat. The action of the alcohols was attributed to the fact that they compete for the polar side-chains of the protein, replacing the removed fat. It was also observed that alcohols increase the hydrophobic properties of the surface of the fat globules and this is considered as the first stage in demulsification.

The Influence of the Physical State of Butterfat on the Body and Texture of Ice Cream

It has been found that the texture of ice cream is influenced by many factors, such as the dispersion of the unfrozen material, ice crystals and air cells. King (1950a) stated that the components which make up the structure of ice cream are separated by interfaces, namely: 1) the air plasma interface which includes the surface of the lamellae, covered by a thin membrane of protein; 2) the fat plasma interface which is covered by a layer of fat emulsifying agents; 3) the ice-plasma interface including ice crystals of various sizes. Cremers (1954) used a microscopical method in ivestigating the internal structure of ice cream. He reported that the fat globules could be detected dispersed in the unfrozen material, around the air cells individually, and in chain-like arrangement. He concluded that the fat globules are orientated, at least partially, around the air cells. This fat-air relationship may vary with different fat contents and percentages of overrun, and may influence the palatability of ice cream. Valear (1960) made a microscopical study of the dispersion of butterfat in the internal structure of ice cream. He classified the states of butterfat dispersion in ice cream into six groups as follows: (a) small homogenized globules,

(b) improperly homogenized globules, (c) small clumps, (d) agglomerates, (e) coalesced globules, and (f) butter granules. He further concluded that an increase in the size of the fat globules occurs during the freezing process. According to Knightly (1959), the physical dispersion of milk fat in the internal structure of ice cream ranges from small, well-dispersed fat globules to visible butter particles. Keeney (1958) reported that over half of the fat in ice cream has been destabilized, in one form or another, after only 20 minutes agitation in the counter freezing. Kloser and Keeney (1958) stated that fat emulsion destabilization or churning often is a very serious defect in operations where a very dry ice cream is being made. They concluded that desirable ice cream dryness is accompanied by some destabilization of the fat in the frozen products. According to King (1950a) the texture of ice cream is related to the finer structure, the size, shape, number, and arrangement of the air cells, ice crystals, lactose crystals, and fat clumps, and the thickness of the foam lamellae. A buttery, greasy texture is due to the presence of lumps of butterfat. Doan (1930) found that with an increase in the percentage of butterfat the instability of protein became more pronounced. This was accounted for by the relationship between the protein

and the salt balance as the fat concentration is increased more protein is adsorbed on the increased surface area of butterfat leaving less protein in the serum to balance the salt.

According to King (1950a) an increase in the fat content of ice cream reduces the size of ice crystals, but there is little relationship between the fat content and the air cells. An increase in the solid-not-fat content reduces the size of both the air cells and ice crystals, through the effect of solids-not-fat in reducing the freezing point. When the fat and the solid-not-fat are properly balanced, these constituents are more effective in reducing the size of air cells and ice crystals. However, Sommer (1951) found that an increase in the fat content of ice cream lead to an improvement in the smoothness of texture by reducing the size of both air cells and ice crystals. Cremers (1954),in his studies of the distribution of milk fat in the internal structure of ice cream, found that milk fat reduced the growth of ice crystals through mechanical obstruction; this was previously observed by Arbuckle (1940). According to Arbuckle the mechanical obstruction by the milk fat permits the water to freeze out of the mix in small ice crystals instead of migrating

to nuclei to form large ice crystals. Cremers (1954) found that increases in the fat content and overrun improved the smoothness of ice cream. He contended that the fat surrounding the air cells was more effective in producing a smooth textured ice cream than the fat dispersed in unfrozen portions. According to Arbuckle (1940) the milk fat and solid-not-fat showed a greater effect on texture than either of these components alone. He also stated that the structure of ice cream was changed with each 2% variation in fat, serum solids and sugar content, and each one-tenth per cent of gelatin in the composition of the mix. Arbuckle (1940) studied the effect of fat content upon texture and structure of ice cream. He reported that there was a highly negative correlation between the fat content and ice crystals size, and a highly positive correlation between the fat content and the distance between ice crystals. He also stated that an increase in serum solids, like fat content, produced smaller ice crystals due to mechanical obstruction. Cole and Boulware (1940) concluded that fat and serum solids content improved the texture and reduced the size of ice crystals. They further stated that fat content in ice cream was found to have a greater effect upon texture judged organoleptically.

Methods of Measuring Destabilized Milk Fat

Quantitative methods for the determination of destabilized milk fat have not been applied extensively, in the past, to ice cream making. The presence of churned or destabilized milk fat has been measured by sensory perception only as the objective was the detection of defective ice cream and not the quantitative measurement of destabilized milk fat.

The Oiling-Off Method.

The oiling-off method has been used for determining the destabilization of the fat emulsion of frozen milk and cream. Webb and Hall (1935) and Bell and Sanders (1945, 1946) used this procedure in their research on frozen milk and cream. Keeney and Josephson (1958) introduced the oiling-off method in evaluating the results they obtained by a turbidity method applied to ice cream. The common procedure is as follows: A 9 gram sample is weighed into a Babcock cream test bottle, and enough water at 140°F is added to bring the level of the liquid to the highest graduation. This test bottle is then placed in a water bath at 140°F for 15 minutes before centrifuging in a warm Babcock centrifuge at 140°F for 30 minutes. The test bottle is then held at 40°F overnight. The warming and centrifuging are repeated next

day, after which the columns of clear fat are read in the usual manner. Holding the samples overnight at $40^{o}F$ after the first centrifuging was found to be necessary in obtaining a clear fat column. Kenney and Josephson (1958) modified the procedure by using an 8 per cent Babcock milk test bottle, centrifuging the sample for 10 minutes and reading the fat column after tempering in a 130 - $140^{o}F$ water bath. Overnight tempering of samples was omitted. With this procedure, two layers are formed in the neck of the test bottle. The top layer is clear butter oil, and below it a "cream-like" layer of partly destabilized milk fat is formed. One-half of the cream-like layer is assumed to be destabilized when measurements are recorded.

The Turbidity Method.

The procedure for the turbidity method, as applied by Keeney and Josephson (1958) to ice cream is as follows: A 1:500 dilution of the mix or the thawed ice cream is prepared by weighing a 1 gram sample into a 50 ml volumetric flask. The weighed sample is diluted to the mark with distilled water, and one millileter of the 1:50 dilution is transferred to a colorimeter tube and diluted with 9 ml. of distilled water using a cream pipette. This 1:500 dilution is then centrifuged for 5 minutes at

1000 r.p.m., and allowed to stand undisturbed for 10 minutes before reading the light transmission with a Klett-Summerson colorimeter fitted with a 500mu light filter. Turbidity is recorded as Klett units. The degree of the fat emulsion stability is calculated as follows, using the turbidity of the mix to indicate 100 per cent stability:

$$\frac{\text{Turbidity of samples (Klett units)}}{\text{Turbidity of mix (Klett units)}} \times 100 = \text{Degree of stability of fat emulsion.}$$

From the values obtained by this formula the percentage of destabilized fat is calculated by subtracting the values from 100. This method is based on the relationship of fat separation with measurable decrease in turbidity as indicated by an increase in light transmission.

The Filtration Method.

Recently, a filtration technique has been reported for assessing the quantity of destabilized milk fat in the high fat content cream used in the Alpha continuous buttermaking procedure. The method developed by Fastova and Vladavets (1955) is as follows: A 5 g sample is weighed into a glass test tube and kept in a water bath for 5 minutes at 65^{o}C. Next, the heated sample is diluted 20 times with distilled water and kept in a cool

water bath at 12°C or less until the fat becomes hard. The cooled dilution is then filtered using a No. 1 glass filter to remove the destabilized portion of the fat from the filtrate. The rate or the degree of destabilization is calculated by using the following formula:

$$D = \frac{F - 20F_f}{F}$$

D = stability of the fat emulsion

F = original fat content of the cream

20 = the rate of dilution

F_f = fat content of the filtrate

Dilution and Separation Method.

This technique was developed by Schulz et al. (1959). The procedure is as follows: Ten grams of the product to be tested are added to 90 ml of tap water at 65°C, and the mixture of the sample and water is kept in a water bath at 65°C for 5 minutes. Next, the warm mixture is transferred to a separatory funnel which is then kept in a water bath at 20°C for 5 minutes. After the 5 minutes cooling, the lower portion is drained and its fat content is determined butyrometrically. The value obtained for the fat content of the lower portion multiplied by 10 indicates the fat content of the cream phase. The term "fat in the cream phase" is to be understood as that cream fat dilutable with temperated water of 65° without the fat being separated.

EXPERIMENTAL METHODS

Composition and Preparation of Experimental Ice Cream.

All the experimental mixes contained 11% serum solids, 15% sucrose (beet sugar), and 0.3% stabilizer-Dariloid K.B.* The milk fat content ranged from 5% to 15%. The emulsifiers, to be described in more detail, were added at 0.1, 0.2 and 0.3%. Stabilizing salts, also to be more fully described, were added at 0.1 and 0.2%.

Some commercial ice creams were examined during the preliminary stages of this investigation.

Sources of Butterfat and Serum Solids.

The fat in the ice cream was obtained from cream and butter oil. The cream, containing approximately 35% butterfat, was obtained from freshly separated, good quality market milk. Fresh butter oil was supplied by a local dairy plant. Additional milk-solid-not-fat was supplied by high heat spray dried skimmilk powder obtained from one source.

Emulsifiers.

The following commercial emulsifiers of known chemical composition were selected for use in the experimental ice creams:

* Made by the Kelco Corporation, 120 Broadway, New York 5 N.Y., U.S.A.

Trade Name	Chemical Composition	
Atmos 150	2/3 mono and 1/3 diglycerides from animal fat.	*
Atmos 300	Mono- and diglycerides	*
Myverol 18-00 (Monoglyceride)	Monoglyceride (fully hydrogenated lard).	**
Myvecet 7-00	Acetylated, hydrogenated monoglyceride (lard).	**
Myverol 18-40	Monoglyceride 40% saturated (palmitic and stearic). 60% unsaturated (oleic and linoleic).	**
Myvetex 8-20	Monoglyceride, 80% Myverol and 20% hydrogenated vegetable oil	**
Span 40	Sorbitan monopalmitate	*
Span 60	Sorbitan monostearate	*
Span 80	Sorbitan mono-oleate	*
Tween 65	Polyoxyethylene sorbitan tristearate	*
Tween 80	Polyoxyethylene derivative of Span - mono-oleate.	*

* Atlas Powder Company Canada Limited

** Distil. Prod. Co., U.S.A.

Emulsion Stabilizing Salts.

In some of the experiments the effectiveness of stabilizing salts was studied. In these experiments two increments of the following salts were used:

1. Sodium hexametaphosphate
2. Sodium oxalate
3. Sodium tetraphosphate.

These salts were added to two series of mixes, one containing Span 60 emulsifier, and the other containing Tween 80 emulsifier.

Processing of Ice Cream Mixes.

The mixes were prepared in 10 pound lots, and pasteurized at a temperature of 160°F for 20 minutes in a home pasteurizer (Saf Gard)*, equipped with an agitator. After pasteurization was completed the mixes were homogenized at 160°F with a Gaulin single stage laboratory homogenizer at 2500 p.s.i. To ensure efficient homogenization, mixes containing butter oil were homogenized twice. Microscopical examination of the mixes indicated that the homogenization was effective. All mixes were cooled and stored at 40°F.

None of the mixes were neutralized. The average

*Pasteurizer is made by the Schlucter Company, Janesville, Wisconsin, U.S.A.

titrable acidity and pH were 0.18% and 6.60, respectively.

Freezing of Mixes and Sampling.

Samples for analysis were taken before freezing, after the freezing temperature (21° - 23°F) and 100% overrun were attained, which hereafter is referred to as the normal freezing time, and after an additional 15 and 30 minute period of agitation in a Taylor-Gilson counter freezer of one gallon capacity. All samples were stored in a hardening room maintained at -7° to -10°F until analyzed. Tests for destabilized butterfat were done in quadruplicate; all other tests were done in duplicate. Results reported in Tables 6 to 10 are averages of three replicates each.

Method for Determining the Destabilized Butterfat in Mixes and Ice Creams.

In the selection of a procedure for measuring the destabilized butterfat content of mixes and ice creams the oiling-off method used by Bell and Sanders (1945), the dilution and filtration method of Favstova and Vladavets (1955), the dilution and separation method of Schulz et al. (1959) and the turbidity procedure of Keeney and Josephson (1958) were compared in some preliminary experiments on commercial mixes and the ice creams made from these mixes. Some of these comparisons are summarized in Table 1.

TABLE 1. A comparison of testing methods applied to mixes and corresponding ice cream.

	Destabilized Fat (%)		
Butterfat %	Oiling-off Method*	Dilution Method Schulz *et al.* (1959)	Dilution and Filtration Method Favstova and Vladavets (1955)
	Tests on Mixes		
10.5	2.5	12.4	11.4
12.6	2.5	13.1	12.3
15.6	3.0	14.4	21.0
	Tests on Ice Creams		
10.5	11.2	12.7	22.8
12.6	11.4	13.1	24.0
15.6	11.5	14.5	27.3

* The tests on mixes did not produce a clear column of destabilized butterfat; a cream-like, partially destabilized layer appeared instead. In the ice cream tests both a clear and a creamy layer were formed; both are included in the results reported.

These results show that for ice cream mixes the dilution methods produced comparable results with the possible exception of the results for the mix testing 15.6% butterfat. For the ice creams comparable results were obtained with the oiling-off method and the method of Schulz *et al*. (1959), however, the results obtained by the procedure of Favstova and Vladavets (1955) were much higher. In this method dilution water at a temperature of 65°C is apparently quite satisfactory for measuring the destabilized butterfat of cream for which the test was developed, but it produces further destabilization when applied to ice creams. Another disadvantage of this procedure is that it requires a filtration under vacuum through a glass filter to speed up the filtration process. Consequently, the filtration procedure of Favstova and Vladavets (1959) was not used.

The remaining methods were compared by testing an ice cream mix that was subjected to freezing and mechanical agitation in the freezer over a considerable period of time. The results of this comparison are summarized in Table 2.

TABLE 2. A further comparison of testing methods in measuring the destabilized butterfat of 13% butterfat ice cream mix subjected to agitation in the freezer.

Time in Freezer Minutes	Temperature F°	Destabilized Fat (%) Turbidity Method	Oiling-Off* Method	Dilution** and Separation Method
0	42	0.01	2.8	4.7
2	26	6.2	2.8	4.7
4	25	21.9	28.7	4.8
6	24	21.3	17.1	5.2
8	23	64.7	19.4	8.8
10	22	75.5	26.2	21.1
12	22	75.5	39.5	28.3
14	21	75.5	34.9	29.2
16	21	76.1	50.5	47.3
18	21	78.2	65.0	54.1
20	21	78.8	72.2	58.3
22	21	78.4	73.4	61.2
24	21.5	79.4	75.4	65.2
26	21	83.8	79.2	67.8
28	21	86.3	79.2	67.9
30	21	86.6	82.3	71.0
32	22	93.1	83.8	72.2
34	22	90.2	85.4	75.2
36	21.5	90.2	86.0	78.5
38	21	90.3	89.2	83.6

* Includes both liquid fat and creamy-like partially destabilized columns.

** Modified by changing dilution from 1:10 to 1:5 and lowering the temperature from 65°C to 38°C.

The results reported in Table 2 show that the percent of destabilized butterfat obtained by the turbidity method was much higher than that obtained by the oiling-off method. Since the values obtained by the oiling-off method include both the clear fat column and the creamy, partially destabilized layer, it is evident that the measurements obtained by the turbidity method do not represent the actual amount of destabilized fat. For this reason the turbidity method was not considered further.

The dilution method of Schultz et al. (1959) was therefore selected as the best available procedure for measuring the destabilization of butterfat in this research. It has the advantage of being based on the determination of the non-destabilized butterfat of the serum of ice cream, which can be accurately determined by the Mojonnier ether extraction method, and the whole procedure is fairly simple.

The accuracy of the method in measuring the destabilized fat content of cream and ice cream mix was assessed by adding butter oil (99.8% destabilized) in measured amounts and checking for the presence of free or destabilized fat. The results of these tests are summarized in Table 3. These data show that the dilution method of Schulz et

al. (1959) did not quantitatively recover all the added butter oil. This was not surprising, as some re-emulsification of the oil undoubtedly occurred, and the large surface area presented to the homogenized butterfat permitted some adsorption of the added butter oil which was not recovered. While the quantitative measurement of the destabilized butterfat is desirable, it was not essential in this investigation. Reproducibility of results is much more important. The results reported in Table 4 show that the variability of the method is within acceptable limits, except the values for sample A which differ more than four times from the mean deviation.

TABLE 3. Recovery of added destabilized butterfat (butter oil) by the Schulz *et al*. (1959) dilution and separation method.

% Butter Oil Added	% Recovery From Cream*	% Recovery From Ice Cream Mix**
5	72.0	79.0
10	78.0	82.0
15	78.3	88.0

* 10% homogenized cereal cream.

** 16% ice cream mix.

TABLE 4. A comparison of the means of the milk fat percent in the (non-destabilized) cream phase obtained from the same ice cream mix.

Samples	Fat Content%*	Standard Deviation of the Means
A - 25 replicates	9.40	± 0.80
B " "	9.62	± 0.58
C " "	9.80	± 0.62
D " "	9.78	± 0.60

* The modified procedure of Shulz et al.(1959).

The Dilution and Separation Method of Schulz et al. (1959) as Modified and used in this Investigation

This method was modified in two respects.

1. Throughout this research a 1:5 dilution was used instead of 1:10 dilution specified in the original procedure. This was justified by the fact that the fat content of ice cream is less than that of the high fat content cream for which this method was originally developed.

2. The temperature of the dilution water was reduced from the recommended 65°C to 38°C. This was done for the following reasons:
   (a) It was found that 65°C dilution water caused additional destabilization of the butterfat.
   (b) This is approximately the body temperature and the milk fat emulsion is quite stable at this temperature.
   (c) This temperature is commonly used in the washing technique for studying the membrane material of the milk fat globule.

Procedure

A 10 g sample is weighed into 100 ml beaker and

diluted with 40 ml of water at 38°C. The diluted sample is left undisturbed for 5 minutes before being transferred to a separatory funnel. The separatory funnel and its contents are placed into a water bath at 20°C for 5 minutes. At the end of the cooling period the separatory funnel was removed from the water bath and the lower portion (bottom layer), amounting to about 35 to 40 ml, is drained into a beaker for fat determination. In most cases, two distinct layers are formed, and where separation is not complete, the addition of 2 ml of 50% ethyl-ether and alcohol was found to improve the separation.

Method of Calculation:

The fat content of the mix and the bottom layer of the mix and the corresponding ice cream was determined* The average fat content of the bottom layer of each dilution was multiplied by 5 (the rate of dilution 1:5). The difference between the fat content of the mix and that of its bottom layer represents the destabilized fat content of the mix. Similarly, the difference between the fat content of the mix and that of the bottom layer of the corresponding ice cream is the destabilized fat content of the ice cream, which includes the destabilized fat content of the mix.

* All fat tests were determined by the Mojonnier method.

Example of Calculation of the Extent of Destabilization.

If an ice cream mix containing 10% butterfat is diluted 1:5 and the bottom layer examined and found to contain 1.96% butterfat, and the value for the corresponding ice cream found to be 1.80% butterfat, then the amount of destabilized fat would be calculated as follows:

The butterfat content of the bottom layer of the mix is

$$1.96 \times 5 = 9.8\%$$

The destabilized fat in the mix is

$$10 - 9.8 = 0.2\%$$

The butterfat content of the bottom layer of ice cream is

$$1.8 \times 5 = 9.0\%$$

The destabilized fat in the ice cream including that of the mix is

$$10 - 9 = 1\%$$

The destabilized fat in the ice cream only is

$$1 - 0.2 = 0.8\%$$

The destabilized fat of the ice cream in percent is

$$\frac{0.8 \times 100}{10} \text{ or } 8\%.$$

Microscopical Examinations.

Mixes and ice creams were examined under the microscope to obtain information on the internal structure, and particularly to observe the changes produced by the experimental procedures. The procedures used were as follows:

(1) Examination of melted samples under ordinary light.

(2) Polarized light microscopy.

(3) Electron microscopy.

Examination of Melted Samples.

The procedure used was a modification of a method developed by Valear (1960). The procedure adopted was as follows: The mixes and melted ice cream samples were diluted 1:100 by adding 0.1 ml of the samples to 10 ml of a 30% glycerine in water solution. One drop of this dilution was placed on a slide with a concavity 10 microns deep and covered with a cover slip. The samples were then examined under the oil immersion objective. The following stains were used in an attempt to color the butterfat to distinguish it from the other constituents: Beta carotene beadlets, Nile blue, bacto-neutral red and Sudan III. The last two, when prepared according to the recommendation of King (1955b) gave the best results when used at the rate of 1:1 with the diluted samples.

Examination by Polarized Light.

Thin sections of ice cream were prepared with a razor blade in the hardening room at -7°F. The sections were mounted on a special slide with a 10 micron deep concavity, and covered with a drop of acetone (refractive

index 1.357). The preparation was then covered with a cover slip and examined under the 50x objective of a Zeiss-Winkel Standard polarizing microscope at 40°F. Photomicrographs were made with the aid of a Zeiss-Winkel attachment camera and focusing eye piece. The magnification, including printing enlargment, was about 750x.

Electron Microscope Examination.

The procedure followed in the preparation of samples for electron microscope observation was similar to that reported by Knoop et al. (1959). Departures from this procedure were as follows:

1. Solution no. 1 was prepared by mixing 80% of butyl metacryalate with 20% of methyl metacrylate. The residue from the prepared specimen was treated with 5 ml of this solution for 30 minutes.
2. Solution no. 2 was prepared by adding 2% benzoic peroxide with solution no. 1 and the residue from the prepared specimen was treated with 5 ml of this solution for 30 minutes. The treatment is then repeated and the residue is left in contact with the solution overnight.
3. The following day a quantity of solution no. 2 was heated to 90°C for 10 to 15 minutes until it starts to become viscous. A gelatin capsule was filled with some of the viscous solution and a sample of the residue was inserted. The capsule was covered

and stored in an oven at 30°C for 48 hours, or until it hardens. The hardened specimens were then ready for the preparation of sections. The sections were mounted on carbon-coated grids for examination. The magnification of the preparations was about 30,000x. The electron microscope used was a Philips EM-200.

RESULTS

Destabilized Fat in Commercial Ice Cream.

Samples of soft-serve and packaged ice cream were collected from local stores and serving places, and examined for the quantity of destabilized fat. Results obtained in this survey are reported in Table 5 which shows a wide variation in the quantity of destabilized fat in soft-serve ice cream. These results also indicate that the problem of fat destabilization is more serious in the soft-serve ice cream than it is in ice cream that is hardened after freezing, as is normal in the ice cream industry. The prolonged agitation of the soft-serve mixes in the freezer is undoubtedly the principal cause for high fat destabilization.

TABLE 5. Percent of destabilized fat in commercial soft-serve and packaged ice cream.

Sample No.	Fat Content	Destabilized Fat (%)
	Soft-Serve	
1	5.0	100.0
2	10.0	80.0
3	9.5	71.6
4	9.8	18.2
5	8.6	30.2
	Packaged Ice Cream	
1	13.2	8.7
2	12.0	9.3
3	12.0	8.8
4	13.5	10.6
5	12.0	9.8
6	12.5	11.5

The Influence of Composition and Processing.

Data have been obtained from many experiments in which the butterfat of the samples varied from 5 to 15%, and in which a number of different emulsifiers were incorporated, and variation in the agitation given ranged from that in the normal freezing procedure to an additional agitation of 15 and 30 minutes after normal freezing. Results from these experiments are summarized in Tables 6 to 10, and in Figs. 1 to 10. Statistical analyses are contained in Tables 1 and 2 of the appendix.

Influence of Fat Content.

Destabilized fat was present in all mixes. The quantity increasing significantly (1% level) as the butterfat content was increased from 5 to 15% (Tables 1 and 2 Appendix). In the ice creams extensive destabilization of the butterfat occurred during the normal freezing operation, and substantial additional destabilization took place in all ice creams as the freezing operation was prolonged. These results were highly significant. Examinations of the data also show that the highest rate of destabilization increase occurred during the freezing operation. The effect of each 5% increment in the fat content from 5 - 15% on the extent of destabilization are summarized in Table 11.

Influence of Emulsifiers.

The destabilization that occurred as a consequence

of the normal processing of the mixes was apparently not influenced by the incorporation of the emulsifiers (Tables 6 - 10). However, during the freezing and agitation of the ice creams the influence of emulsifiers was apparent. Ice creams containing Tween 80 had a considerably greater quantity of destabilized fat than the control. The emulsifiers Span 60 and Myverol 18-00, on the other hand, reduced the destabilization to a point well below that of the controls. The reduction was particularly marked when the emulsifier Span 60 was incorporated. All of these observations were found to be highly significant. The means of the destabilized fat found in ice creams made from cream with Sapn 60 emulsifier were compared with those made with Myverol 18-00 emulsifier using Duncan's multiple range test, Steel and Torrie (1960). Results revealed no significant difference between the means of the two emulsifiers in mixes of the same butterfat content. This was also true for ice creams prepared from butter oil. However, when the means of the same emulsifiers in ice creams made from cream were compared with those of ice creams made from butter oil, a significant difference was observed in mixes containing 10% or more butterfat. This indicates that the reducing effect of these emulsifiers on the rate of fat destabilization is greater in ice creams made from cream than those made from butter oil when the fat content is 10% or higher.

TABLE 6. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice cream containing 5% butterfat.

Emulsifier Added	Destabilized Fat (%)			
	Mixes	Normal Freezing Time (Ca.10 min)	Additional Agitation	
			15 minutes	30 minutes
Butterfat Source - Cream				
Control	4.9	20.8	22.6	27.3
Tween 80	4.6	32.6	45.8	48.6
Span 60	4.3	17.0	20.6	24.1
Myverol 18-00	4.0	18.0	21.8	26.3
Butterfat Source - Butter Oil				
Control	4.2	18.6	23.0	28.0
Tween 80	5.4	39.6	45.4	60.8
Span 60	5.4	16.0	20.3	24.0
Myverol 18-00	5.2	16.7	22.3	27.5

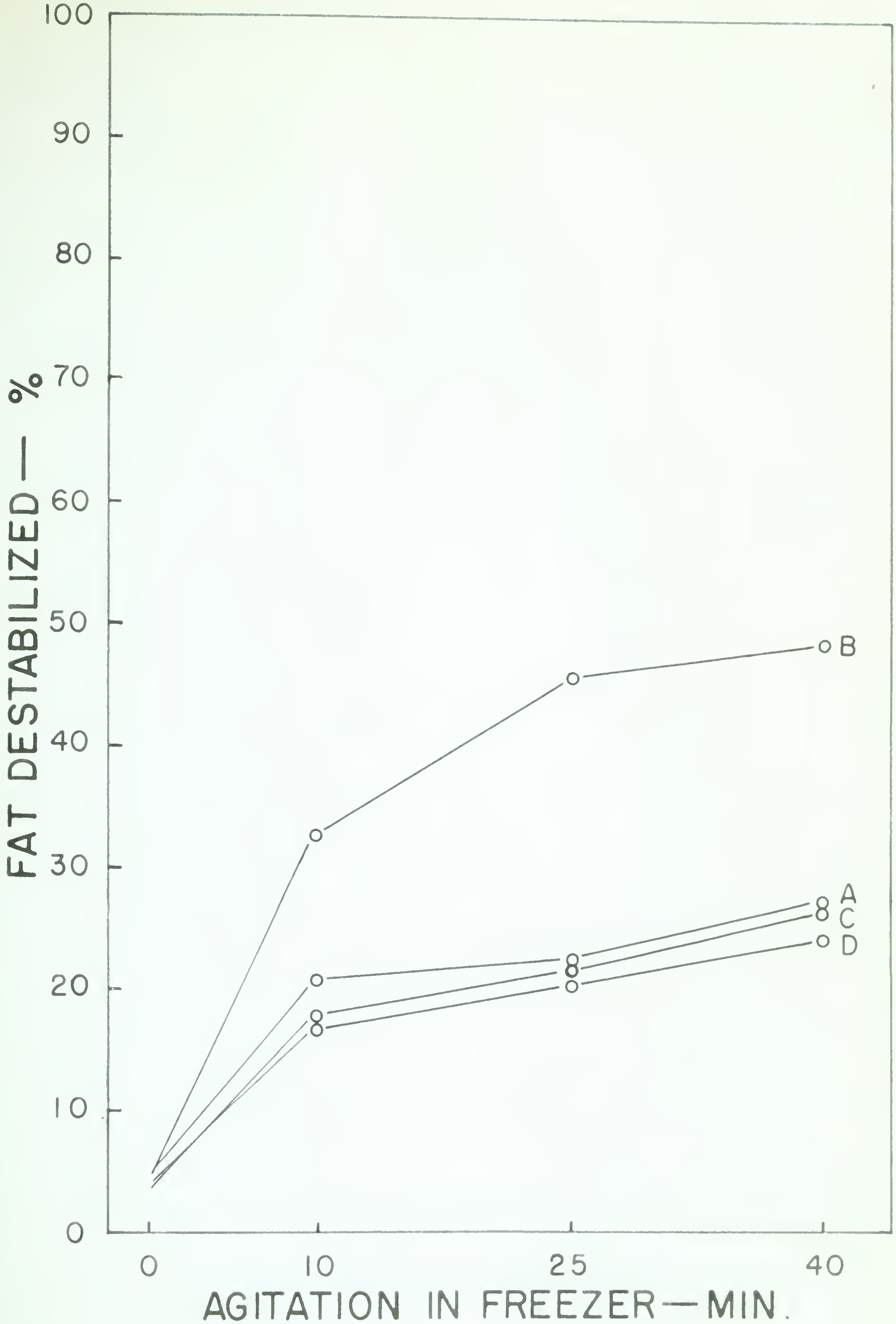

Fig. 1. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice cream containing 5% butterfat (source of butterfat - cream). (A) Control (B) Tween 80 (C) Myverol 18-00 (D) Span 60

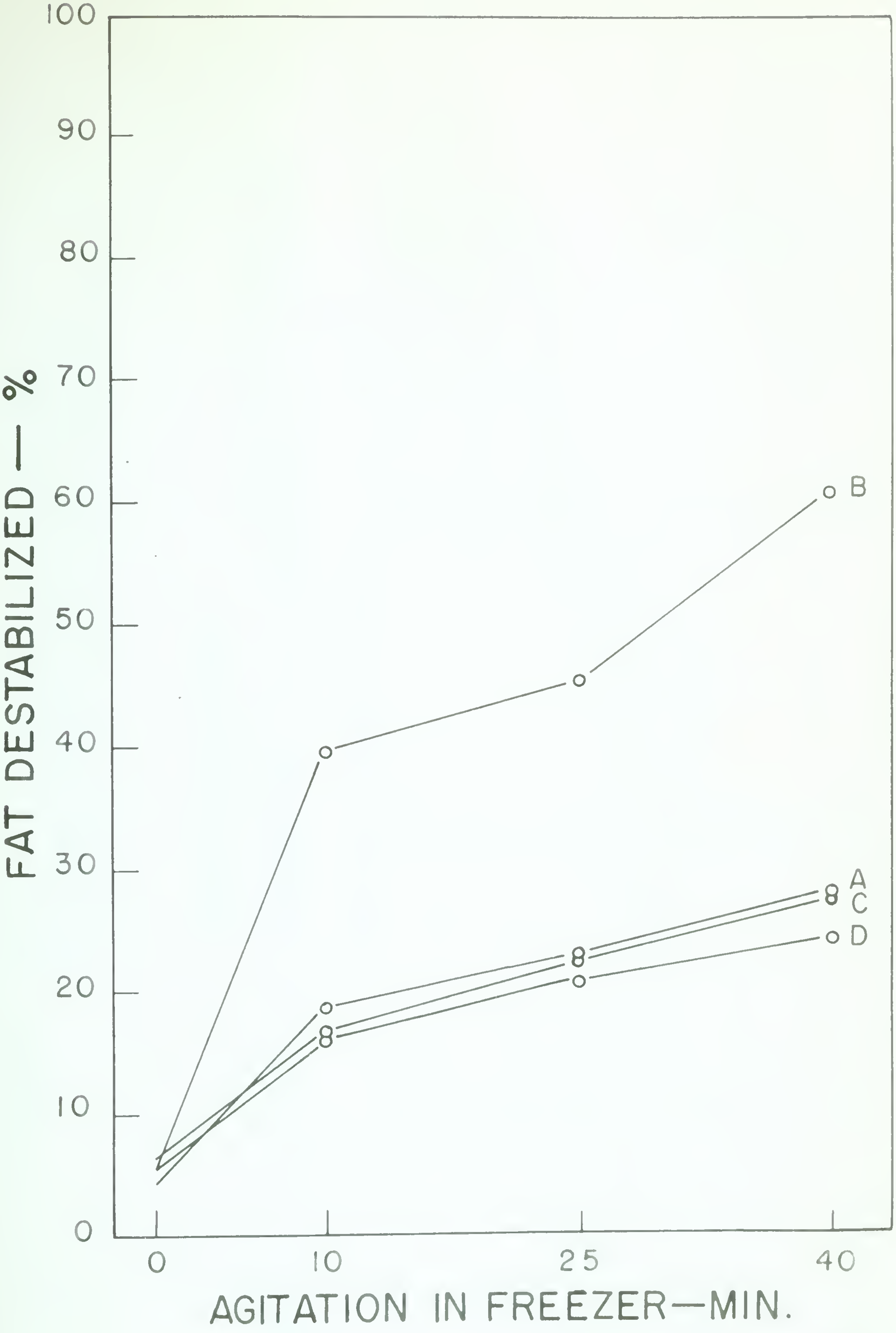

Fig. 2. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice cream containing 5% butterfat (source of butterfat - butter oil). (A) Control (B) Tween 80 (C) Myverol 18-00 (D) Span 60

TABLE 7. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice cream containing 8% butterfat.

	Destabilized Fat (%)			
Emulsifier Added	Mixes	Normal Freezing Time (Ca. 10)	Additional Agitation 15 minutes	Additional Agitation 30 minutes
		Butterfat Source - Cream		
Control	6.0	25.8	26.6	30.5
Tween 80	6.2	42.2	50.0	58.2
Span 60	5.0	21.2	22.6	25.1
Myverol 18-00	5.2	23.5	25.6	27.5
		Butterfat Source - Butter Oil		
Control	6.8	26.1	28.6	32.6
Tween 80	6.8	53.2	60.7	80.6
Span 60	6.1	22.3	24.4	28.5
Myverol 18-00	6.1	25.0	26.0	30.0

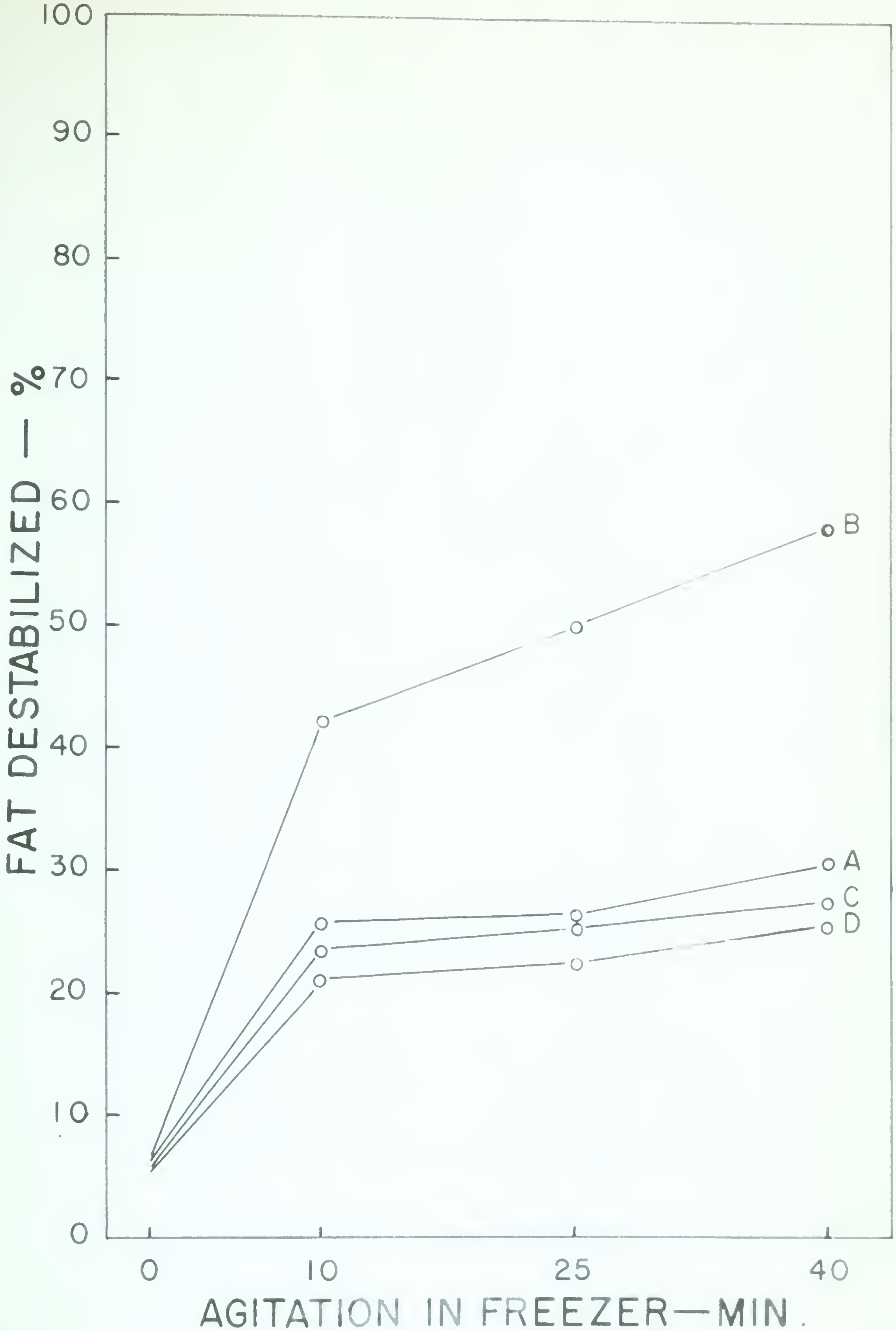

Fig. 3. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice cream containing 8% butterfat (source of butterfat - cream). (A) Control (B) Tween 80 (C) Myverol 18-00 (D) Span 60

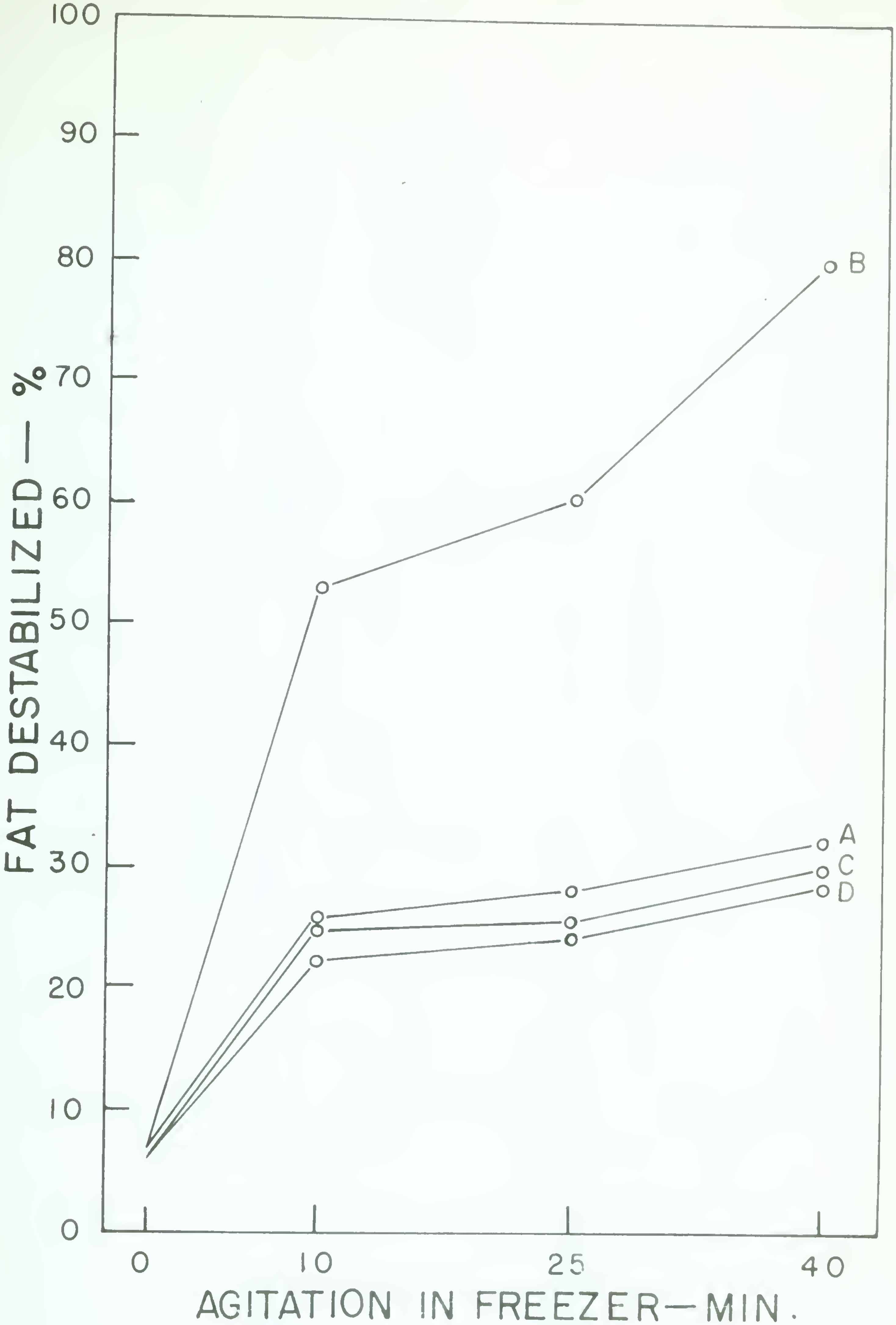

Fig. 4. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice cream containing 8% butterfat (source of butterfat - butter oil). (A) Control (B) Tween 80 (C) Myverol 18-00 (D) Span 60

TABLE 8. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat.

	Destabilized Fat (%)			
Emulsifier Added	Mixes	Normal Freezing Time (Ca 10 min)	Additional Agitation 15 minutes	Additional Agitation 30 minutes
		Butterfat Source - Cream		
Control	6.3	27.6	29.5	38.0
Tween 80	6.6	46.0	51.0	74.3
Span 60	6.2	22.8	24.3	32.0
Myverol 18-00	7.0	23.7	25.5	34.0
		Butterfat Source - Butter Oil		
Control	8.5	30.3	33.2	37.8
Tween 80	9.5	54.2	69.7	80.8
Span 60	9.5	24.8	28.0	30.8
Myverol 18-00	9.0	26.8	29.2	36.8

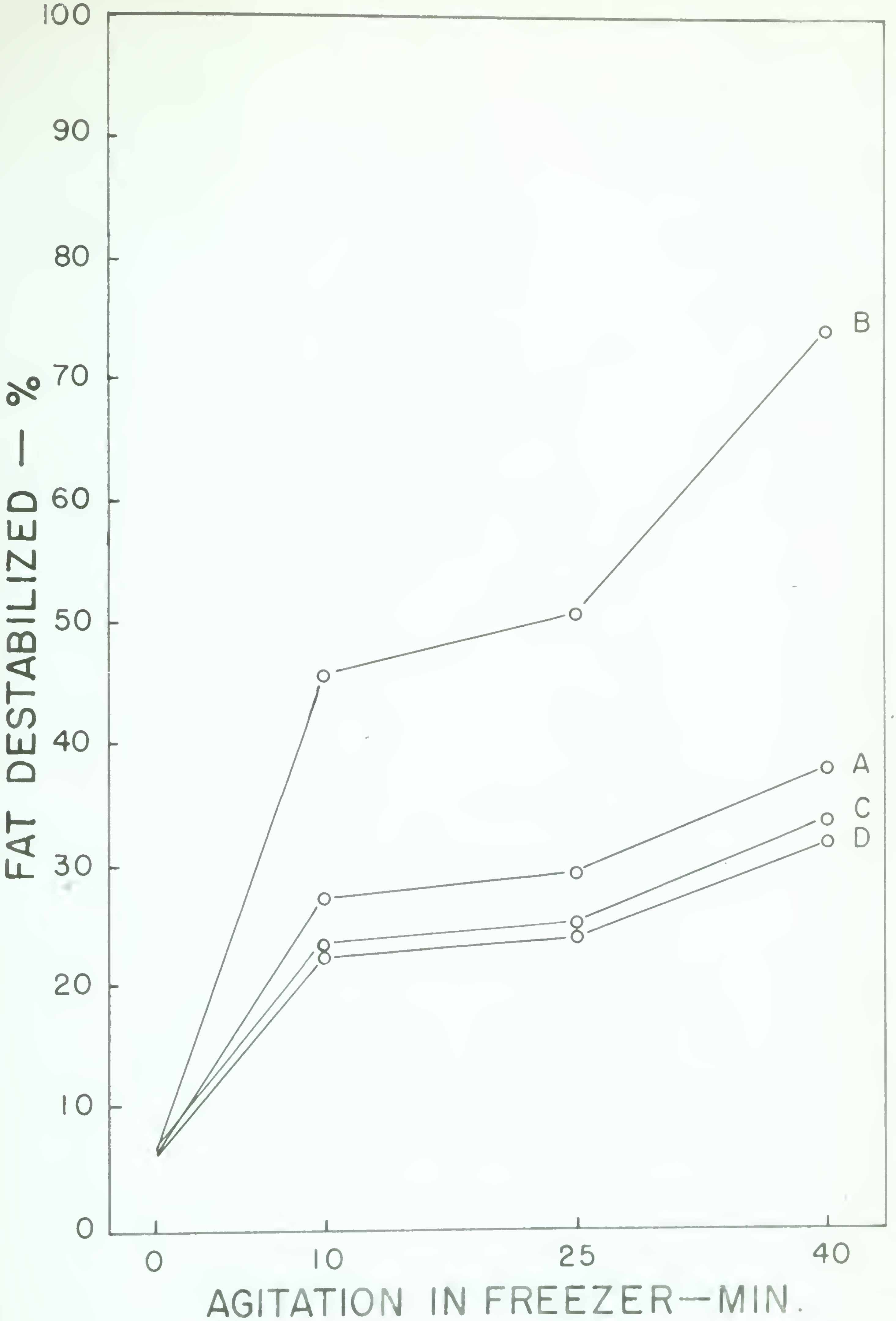

Fig. 5. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat (source of butterfat - cream). (A) Control (B) Tween 80 (C) Myverol 18-00 (D) Span 60

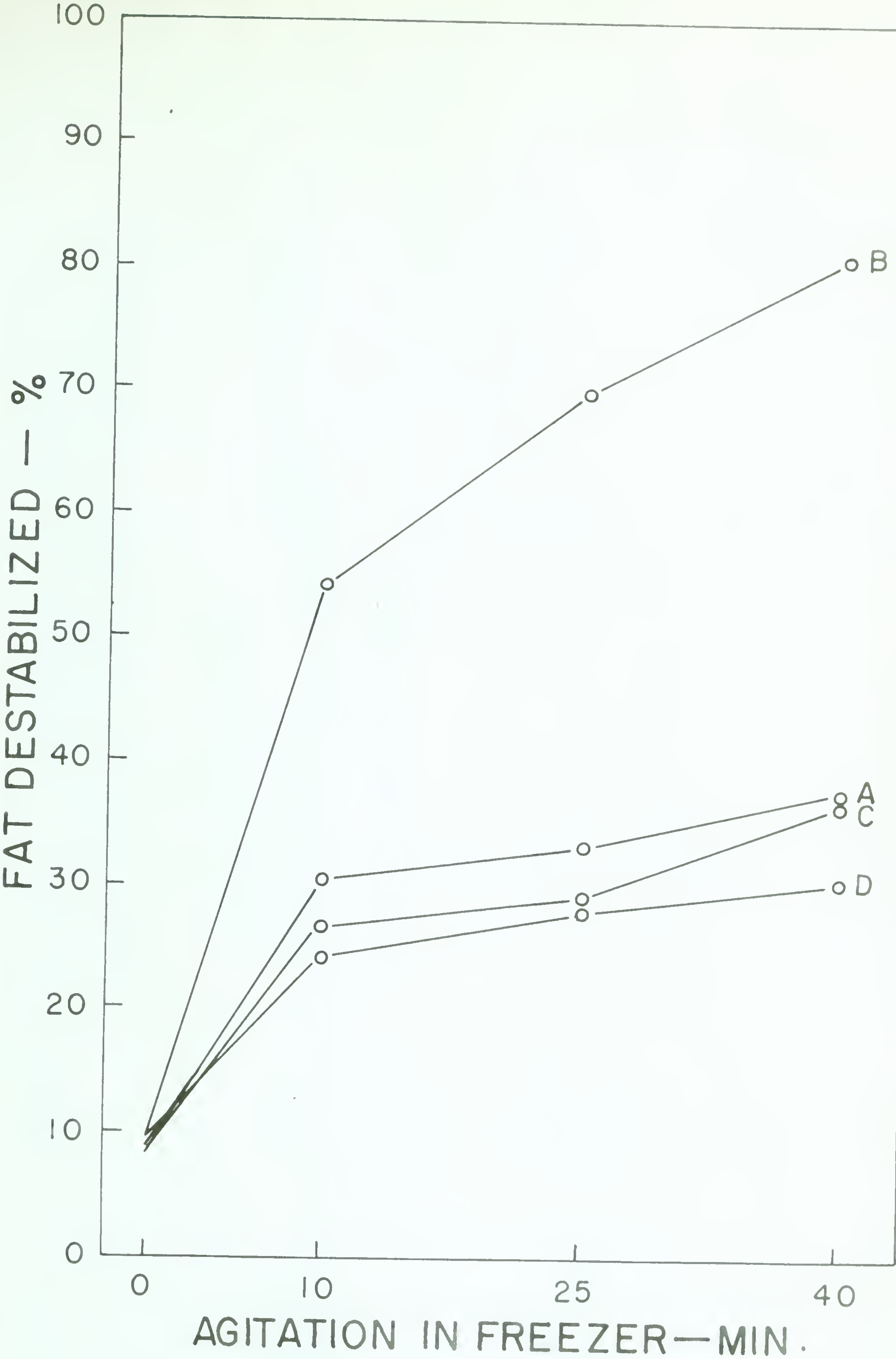

Fig. 6. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat (source of butterfat - butter oil). (A) Control (B) Tween 80 (C) Myverol 18-00 (D) Span 60

TABLE 9. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice creams containing 12% butterfat.

Emulsifier Added	Destabilized Fat (%)			
	Mixes	Normal Freezing Time (Ca. 10 min)	Additional Agitation	
			15 minutes	30 minutes
Butterfat Source - Cream				
Control	8.0	36.9	38.0	39.4
Tween 80	9.0	47.8	53.4	76.8
Span 60	8.0	22.5	28.0	32.0
Myverol 18-00	8.6	30.8	32.2	35.0
Butterfat Source - Butter Oil				
Control	8.8	36.6	39.2	43.0
Tween 80	9.4	54.4	70.1	83.2
Span 60	9.2	24.0	29.5	33.0
Myverol 18-00	9.2	32.0	34.2	36.9

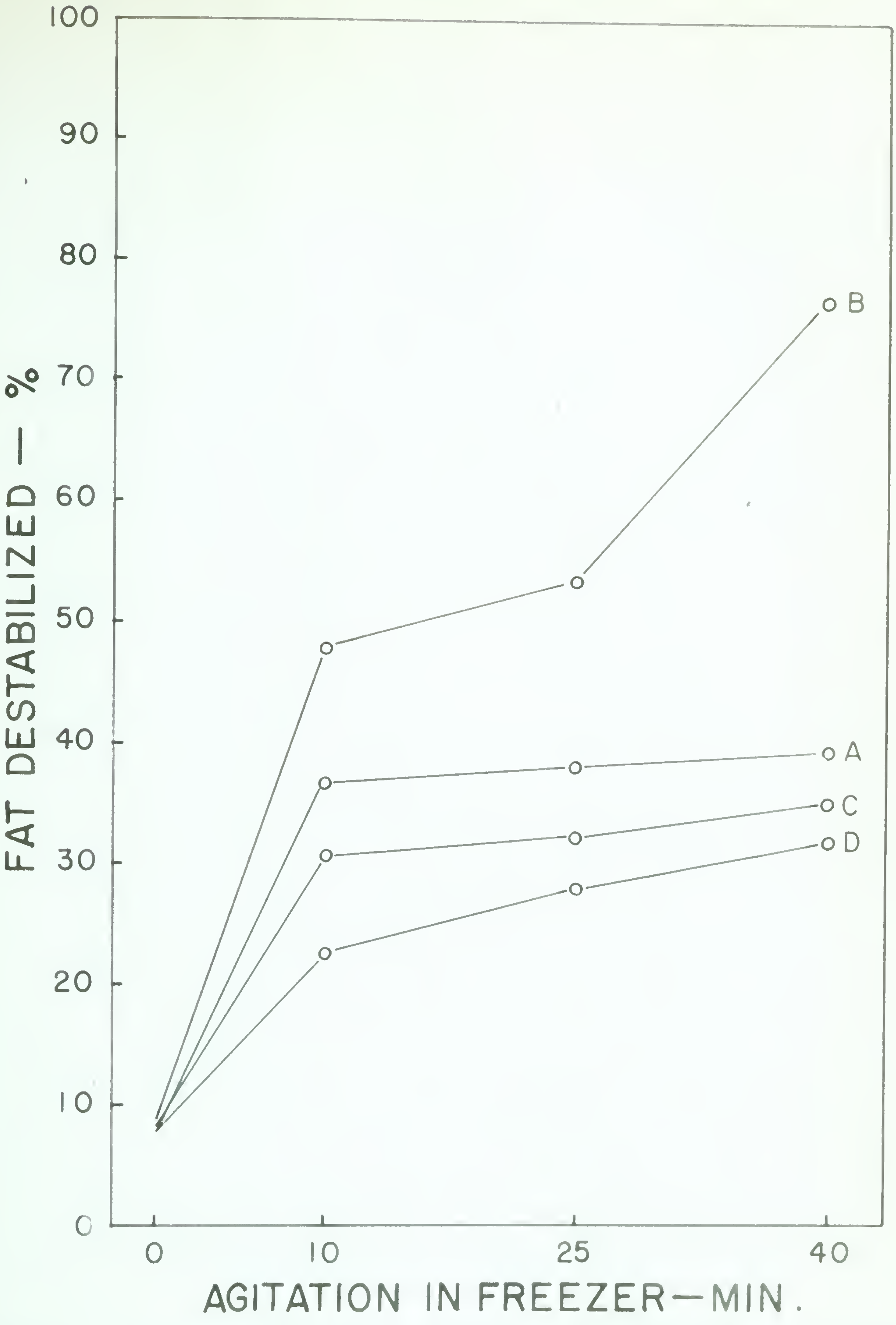

Fig. 7. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice cream containing 12% butterfat (source of butterfat - cream). (A) Control (B) Tween 80 (C) Myverol 18-00 (D) Span 60

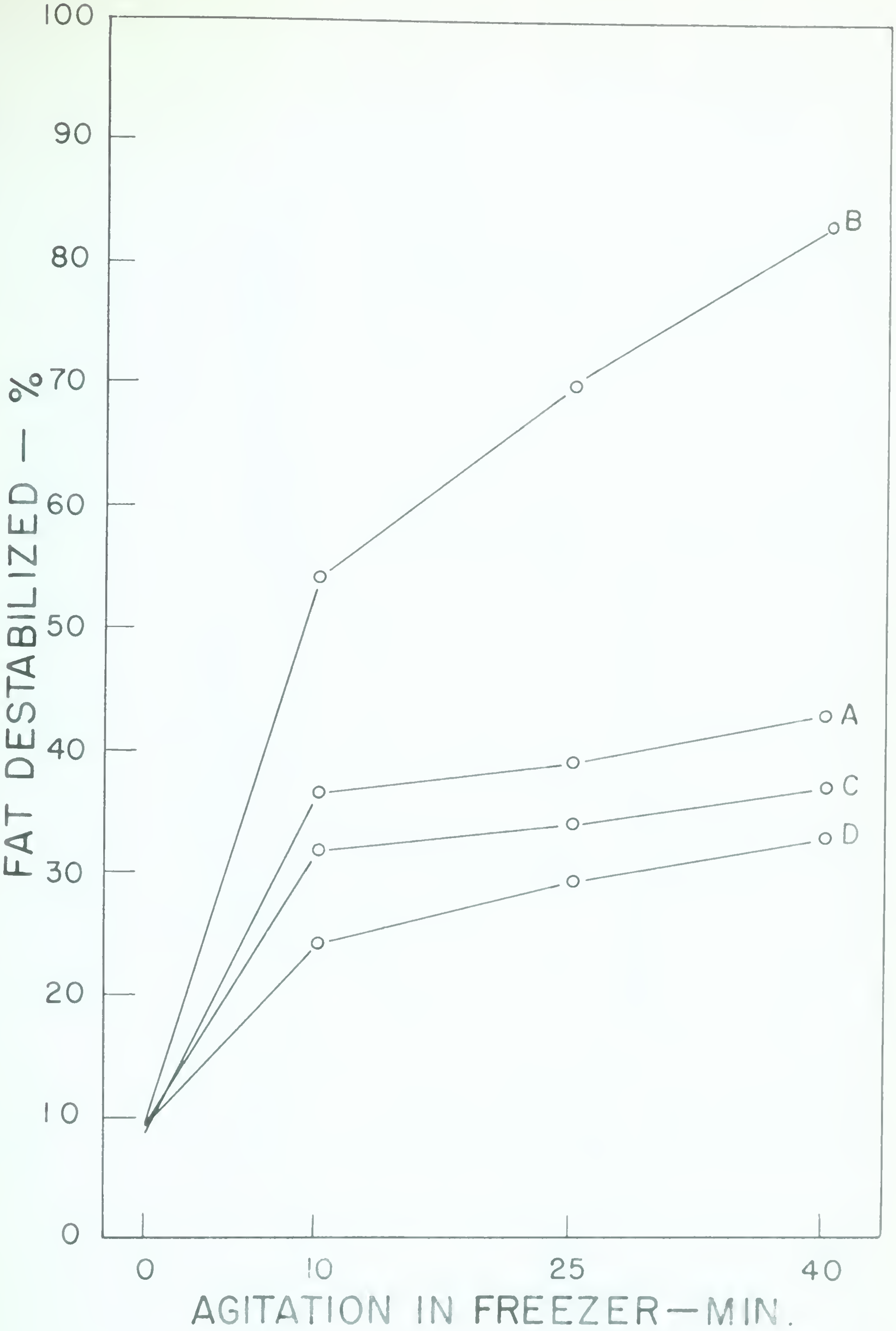

Fig. 8. The effect of incorporation of different emulsifiers and varying degrees of agitation on the dettabilization of fat in mixes and corresponding ice cream containing 12% butterfat (source of fat - butter oil).
(A) Control (B) Tween 80 (C) Myverol 18-00 (D) Span 60

TABLE 10. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice creams containing 15% butterfat.

	Destabilized Fat (%)			
Emulsifier Added	Mixes	Normal Freezing Time (Ca. 10 min)	Additional Agitation 15 minutes	Additional Agitation 30 minutes
		Butterfat Source - Cream		
Control	8.3	39.3	43.7	64.4
Tween 80	8.6	50.5	55.4	85.5
Span 60	8.0	30.3	38.3	45.8
Myverol 18-00	8.2	32.2	40.0	48.3
		Butterfat Source - Butter Oil		
Control	9.8	41.7	48.7	64.9
Tween 80	9.6	58.2	75.8	88.7
Span 60	9.6	35.4	42.0	47.8
Myverol 18-00	9.5	39.7	45.0	51.4

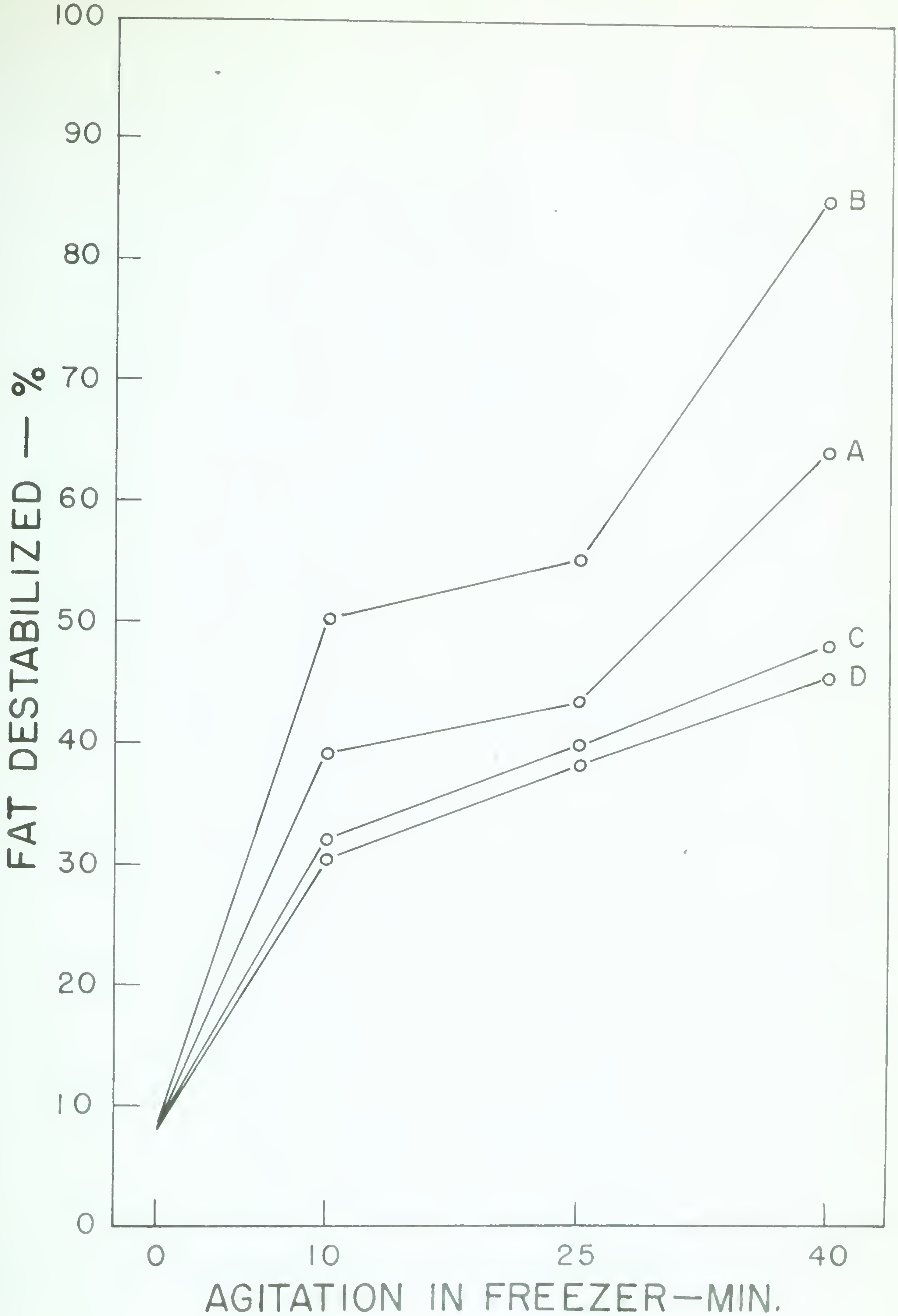

Fig. 9. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice crem containing 15% butterfat (source of butterfat - cream). (A) Control (B) Tween 80 (C) Myverol 18-00 (D) Span 60

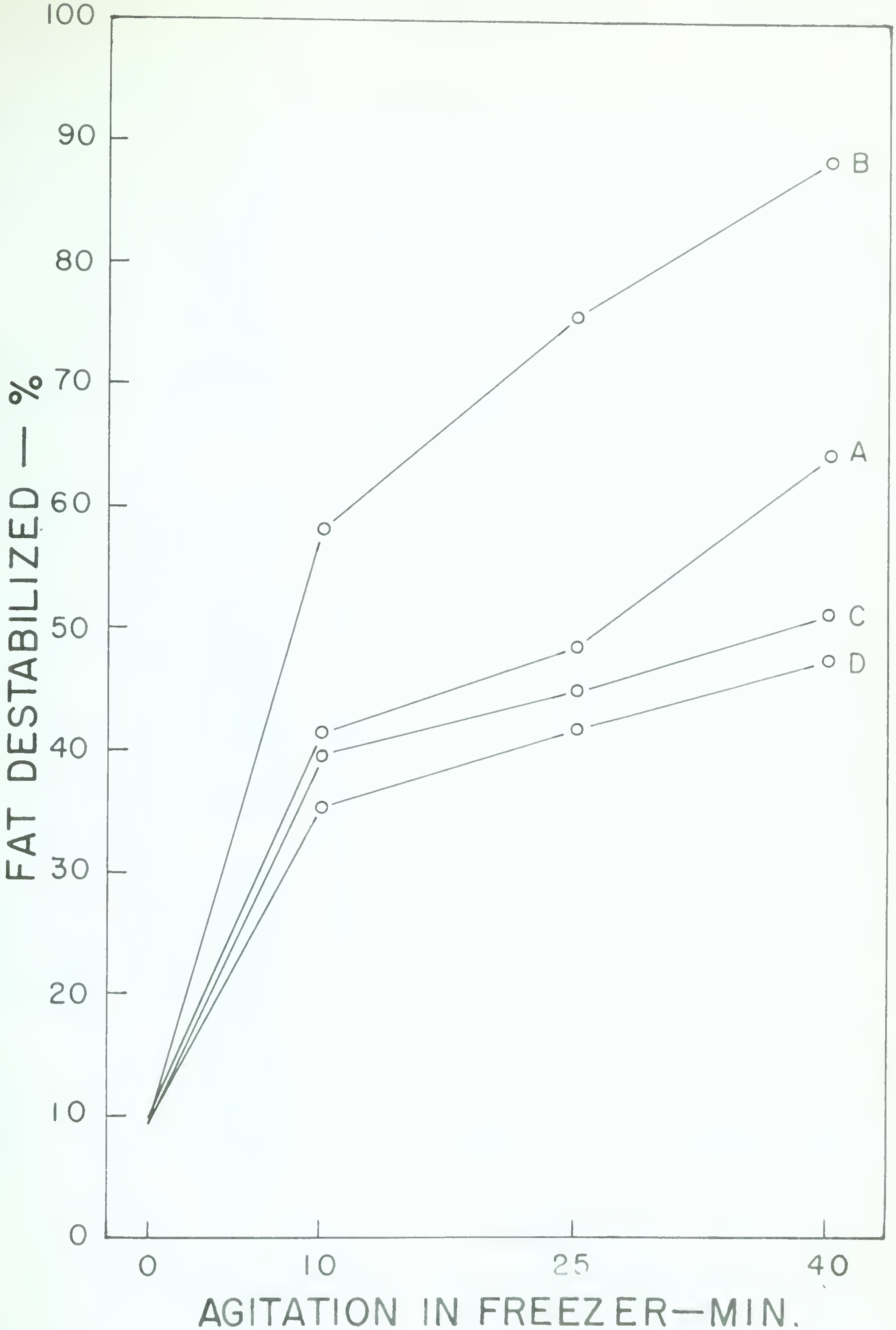

Fig. 10. The effect of incorporation of different emulsifiers and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice cream containing 15% butterfat (source of butterfat - butter oil). (A) Control (B) Tween 80 (C) Myverol 18-00 (D) Span 60

TABLE 11. Increases in the proportion of destabilized fat as a result of 5% increments (from 5 to 15%) of fat in experimental samples of ice cream.

Emulsifiers Added	Increase in Destabilization (%)					
	Normal Freezing Time (Ca. 10 min)		15 Minutes Additional Agitation		30 Minutes Additional Agitation	
	5 - 10%	10 - 15%	5 - 10%	10 - 15%	5 - 10%	10 - 15%
Butterfat Source - Cream						
Control	6.8	11.7	6.9	8.5	16.4	20.7
Tween 80	13.4	4.5	5.2	23.3	6.8	30.1
Span 60	5.8	7.5	3.7	7.7	14.2	7.5
Myverol 18-00	5.7	8.5	3.7	8.5	13.7	8.3
Butterfat Source - Butter Oil						
Control	11.7	11.4	10.2	15.5	9.8	27.1
Tween 80	14.6	4.0	24.3	6.1	20.0	8.9
Span 60	8.8	10.6	7.7	14.0	6.8	17.0
Myverol 18-00	10.1	12.9	6.9	15.8	9.3	14.6

The Stabilizing Influence of Different Concentrations of Three Emulsifiers.

In the data previously reported in Tables 1 - 6, the emulsifiers were incorporated at the concentration of 0.1% normally recommended by the manufacturers of emulsifiers used in ice cream making. As the published literature does not contain sufficient information on the effect of increasing the emulsifier concentration on the destabilization of the fat of ice cream, two series of experiments were set up in which the increments of emulsifiers were 0.1, 0.2 and 0.3%. In one series of ice creams, cream was the source of butterfat and in the other series butter oil was used. The results obtained in these experiments are shown in Tables 12, 13 and 14, and Figs. 11 - 16.

These data show that all three emulsifiers used, Tween 80, Span 60 and Myverol 18-00, caused greater destabilization as the amount incorporated in the ice cream increased from 0.1 to 0.3%. However, the greatest increase in destabilization occurred in the mixes containing Tween 80 (Table 12). The destabilization caused by Span 60 (Table 13), and Myverol 18-00 (Table 14) also increased with increasing concentration of emulsifier, but in only one instance with Span 60 did the destabilization exceed that of the

controls, and that occurred at normal freezing time, (Table 13). Again, as reported above, the greatest rate of destabilization occurred during the normal freezing procedure, but additional agitation over a much longer time than that of the freezing operation increased destabilization in all cases. Increases in destabilization of the mixes did not occur in proportion to the addition of emulsifier. The observations reported here were all highly significant (Table 3 appendix). Application of Duncan's multiple range test (Steel and Torrie, 1960) failed to show any significant difference between Span 60 and Myverol at all fat levels and emulsifier concentrations in both series of ice creams. The difference between these two emulsifiers and Tween 80 in their influence on butterfat destabilization is obvious from an examination of the data.

TABLE 12. The effect of increasing the concentration of incorporated Tween 80 emulsifier, and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat.

Emulsifiers Added Quantity	Destabilized Fat (%)			
	Mixes	Normal Freezing Time (Ca. 10 min.)	Additional Agitation	
			15 minutes	30 minutes
		Butterfat Source - Cream		
Control	7.2	20.4	45.1	61.6
0.1% Tween 80	8.5	50.8	62.8	69.7
0.2% Tween 80	8.4	54.1	72.7	79.0
0.3% Tween 80	8.7	55.8	80.2	89.0
		Butterfat Source - Butter Oil		
Control	10.1	22.6	59.6	70.4
0.1% Tween 80	9.6	50.4	66.3	72.0
0.2% Tween 80	8.5	51.4	77.7	85.2
0.3% Tween 80	9.5	55.1	82.4	87.5

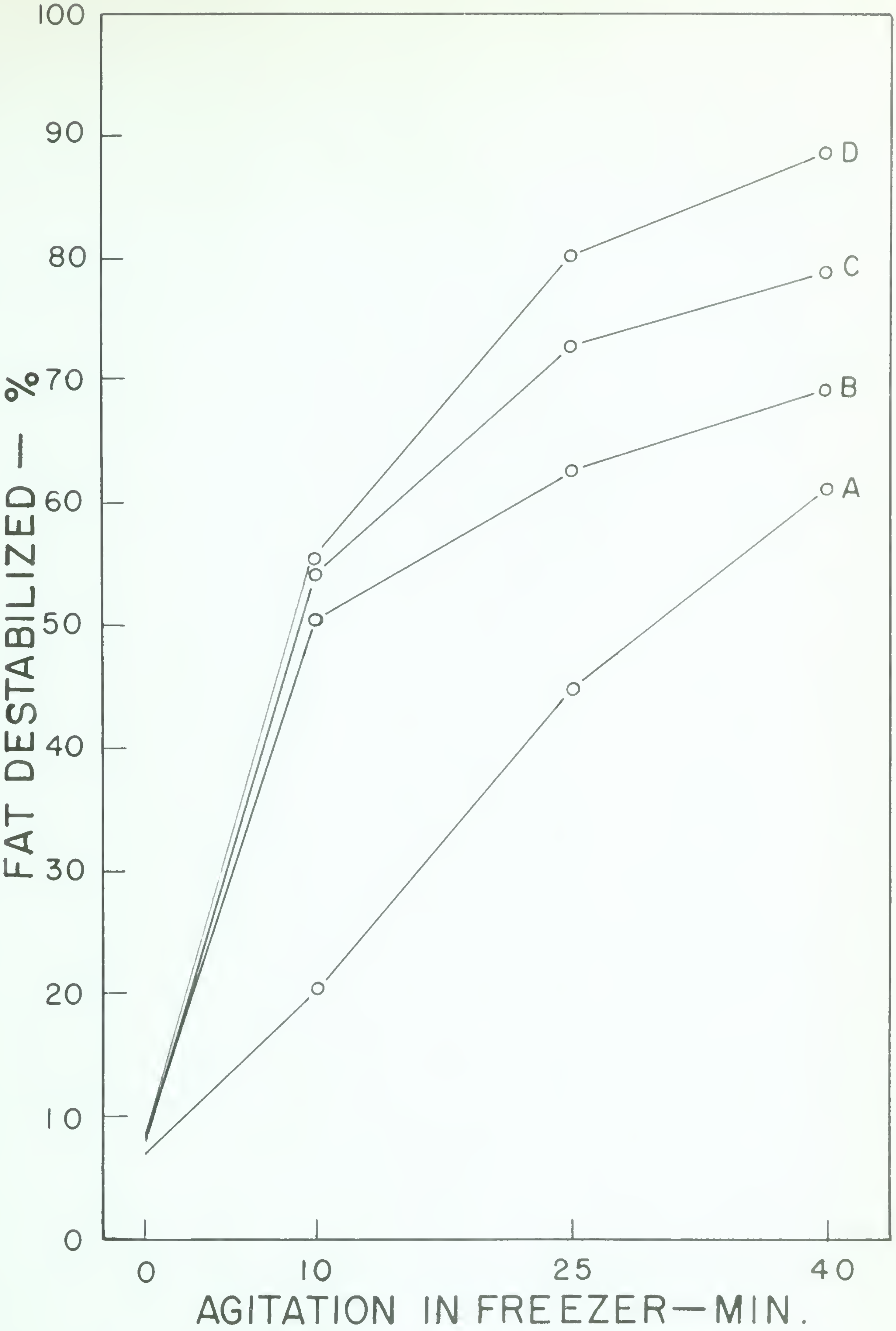

Fig. 11. The effect of increasing the concentration of incorporated Tween 80 emulsifier and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat (source of butterfat - cream). (A) Control (B) 0.1% (C) 0.2% (D) 0.3%

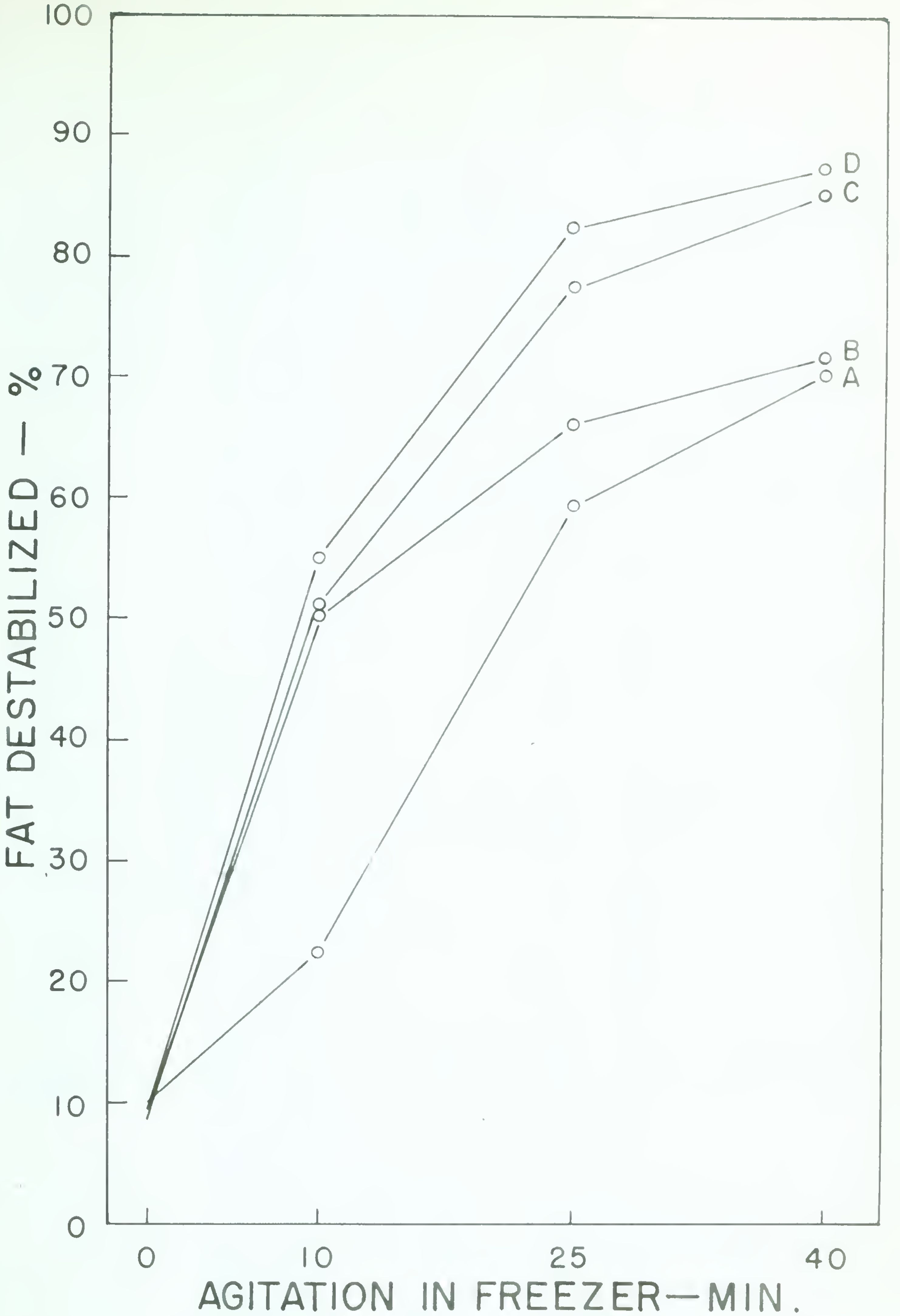

Fig. 12. The effect of increasing the concentration of incorporated Tween 80 emulsifier and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat (source of butterfat - butter oil). (A) Control (B) 0.1% (C) 0.2% (D) 0.3%

TABLE 13. The effect of increasing the concentration of incorporated Span 60 emulsifier and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat.

Emulsifier Added Quantity	Destabilized Fat %			
	Mixes	Normal Freezing Time (Ca. 10 min)	Additional Agitation	
			15 minutes	30 minutes
		Butterfat Source - Cream		
Control	8.4	20.4	45.1	61.6
0.1% Span 60	8.8	18.9	26.1	44.1
0.2% "	7.8	19.2	30.6	46.4
0.3% "	8.9	20.6	32.6	52.7
		Butterfat Source - Butter Oil		
Control	10.1	22.6	59.6	70.4
0.1% Span 60	8.8	15.4	46.4	50.0
0.2% "	8.2	18.9	48.6	54.6
0.3% "	8.4	20.0	52.2	61.7

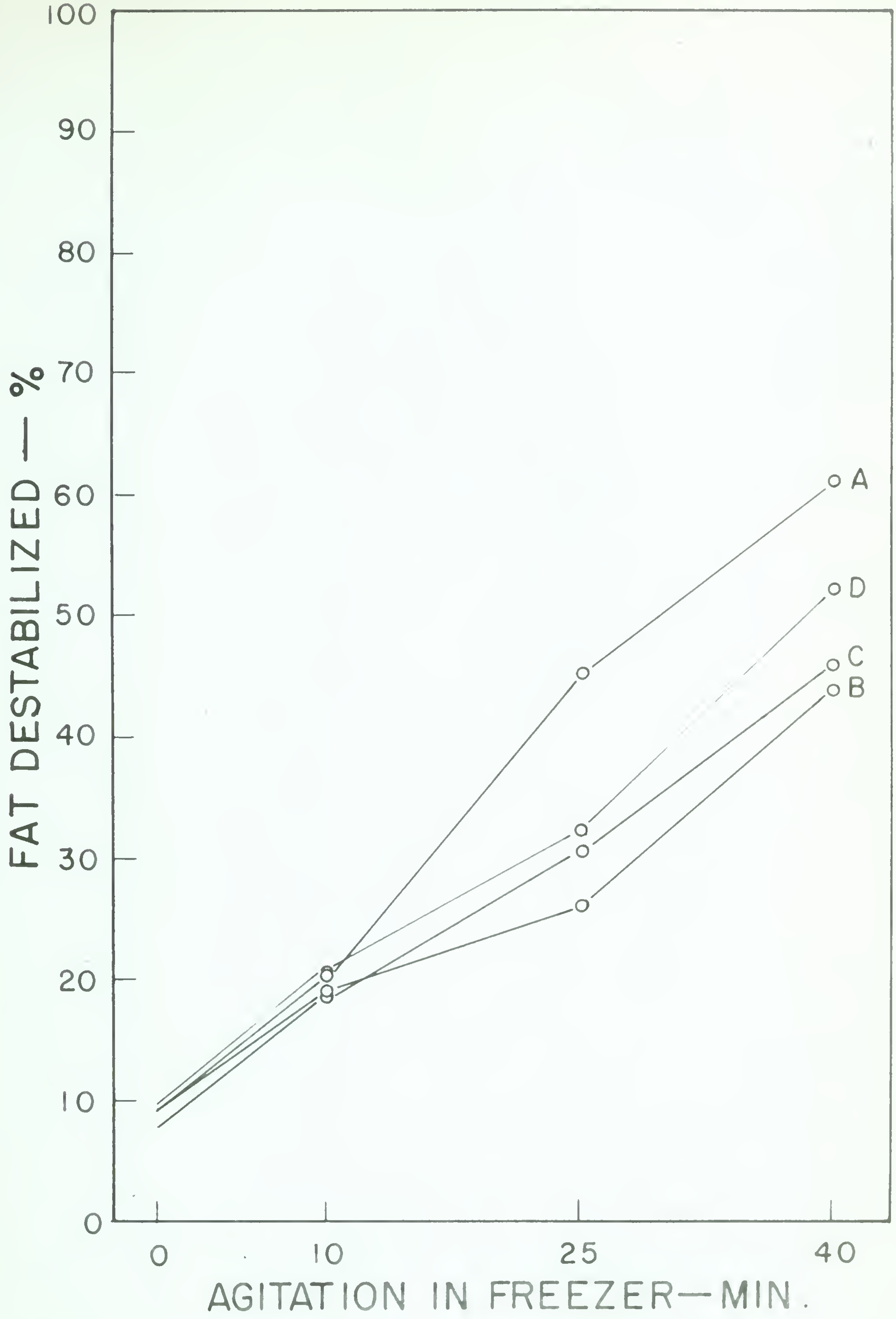

Fig. 13. The effect of increasing the concentration of incorporated Span 60 emulsifier and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat (source of butterfat - cream). (A) Control (B) 0.1% (C) 0.2% (D) 0.3%

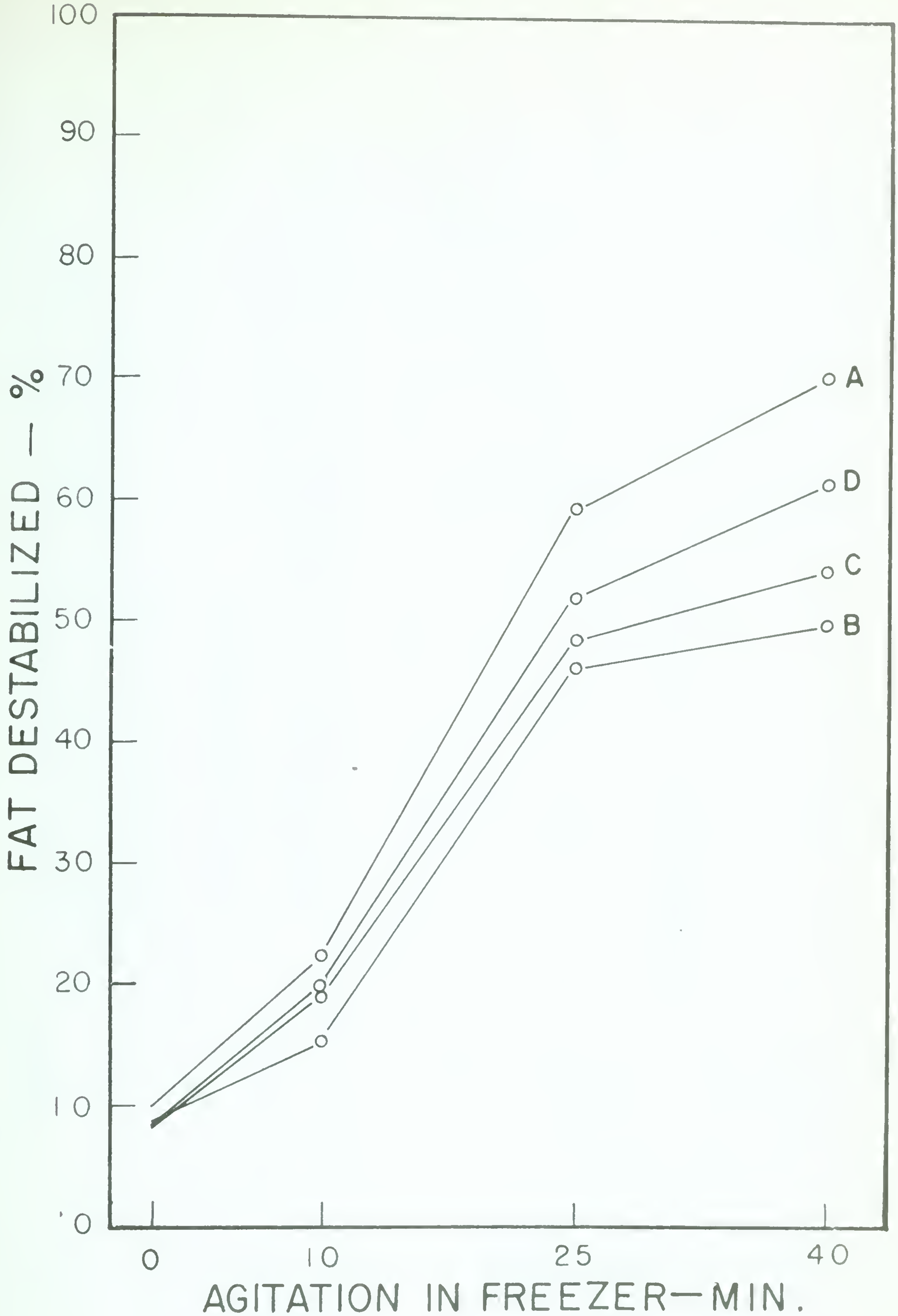

Fig. 14. The effect of increasing the concentration of incorporated Span 60 emulsifier and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat (source of butterfat - butter oil). (A) Control (B) 0.1% (C) 0.2% (D) 0.3%

TABLE 14. The effect of increasing the concentration of incorporated Myverol 18-00 emulsifier, and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat.

Emulsifier Added Quantity	Destabilized Fat (%)			
	Mixes	Normal Freezing Time (Ca. 10 min)	Additional Agitation 15 minutes	Additional Agitation 30 minutes
Butterfat Source - Cream				
Control	8.4	20.4	45.5	61.6
0.1% Myverol 18-00	8.1	16.3	20.3	35.1
0.2% "	8.7	17.2	23.2	39.3
0.3% "	8.3	22.1	33.3	44.4
Butterfat Source - Butter Oil				
Control	10.1	22.6	59.6	70.4
0.1% Myverol 18-00	8.7	19.0	52.2	62.9
0.2% "	9.1	18.6	50.3	56.8
0.3% "	9.5	20.1	55.1	68.2

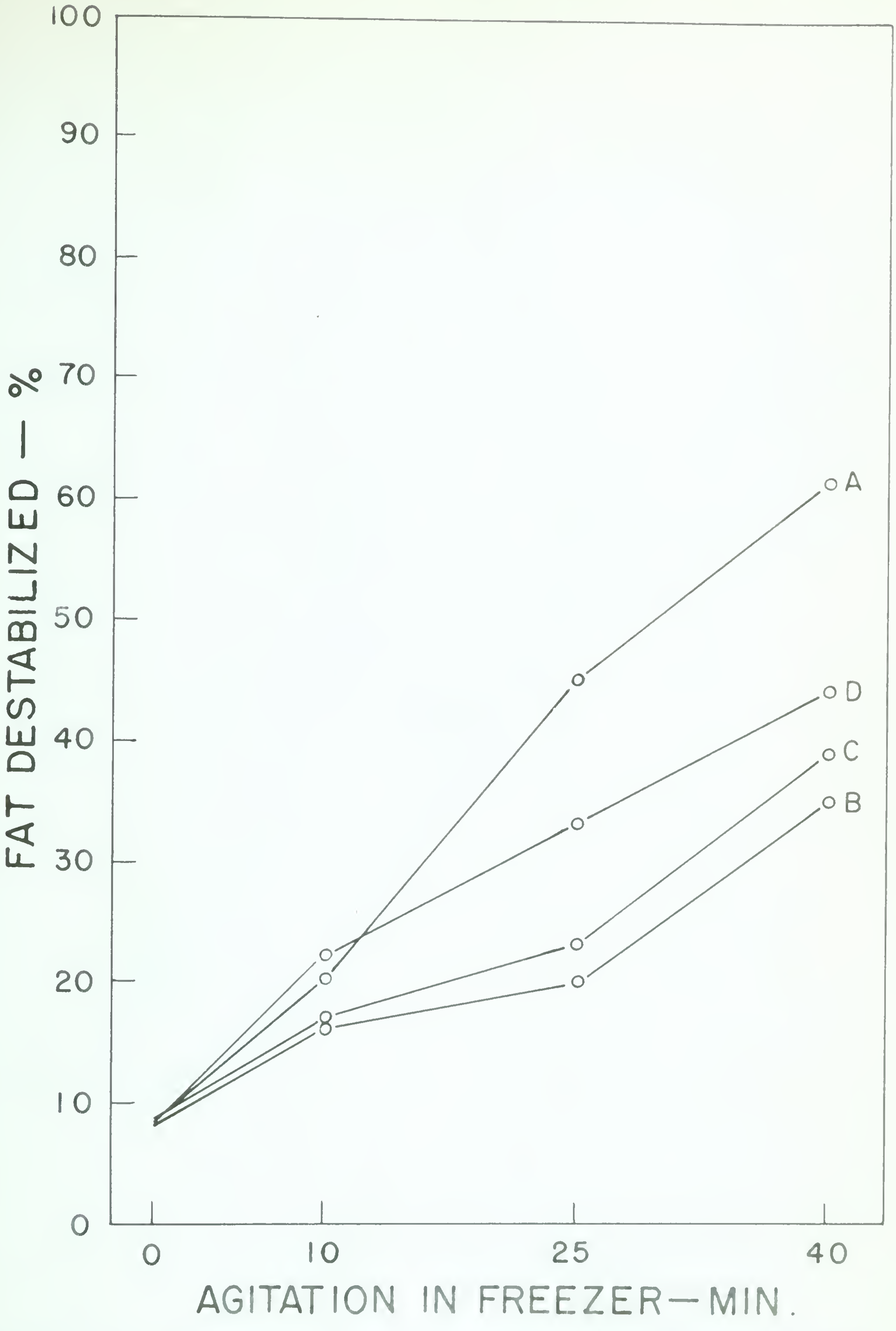

Fig. 15. The effect of increasing the concentration of incorporated Myverol 18-00 emulsifier and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat (source of butterfat - cream). (A) Control (B) 0.1% (C) 0.2% (D) 0.3%

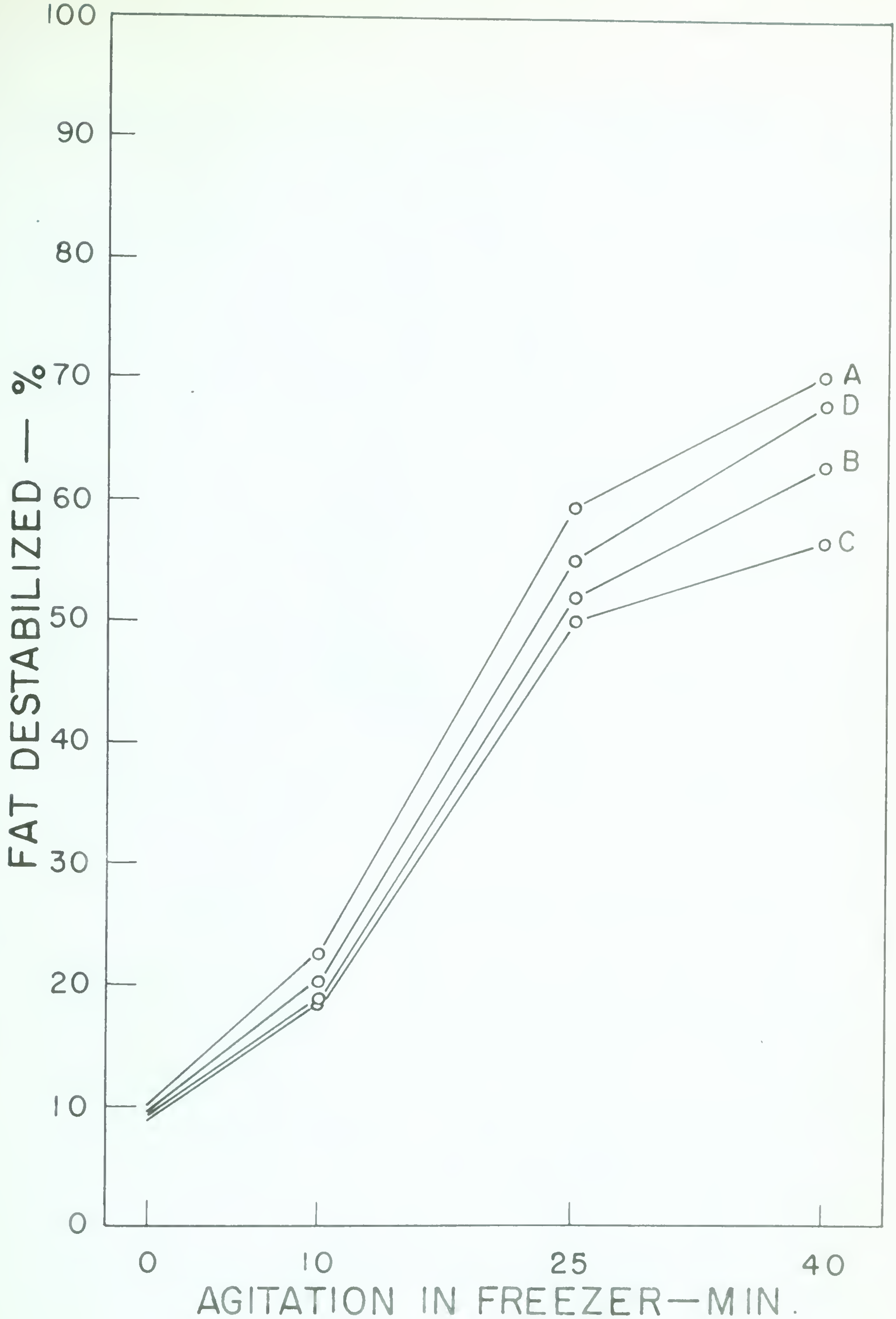

Fig. 16. The effect of increasing the concentration of incorporated Myverol 18-00 emulsifier and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat (source of butterfat - butter oil). (A) Control (B) 0.2% (C) 0.1% (D) 0.3%

The Effect of a Number of Additional Emulsifiers.

In the experiments summarized in Tables 6 - 10 it was necessary to concentrate on only a few emulsifiers because of the large number of mixes that would otherwise have been involved. Consequently, in order to obtain information on the effect of a considerable number of other emulsifiers on the destabilization of the butterfat a series of ice cream mixes (10% butterfat) were made from identical constituents except for the eight emulsifiers which were added in concentrations of 0.1 and 0.2%. The mixes and the ice cream made from them were prepared under the same conditions described earlier, and were analyzed for destabilized butterfat; the results are summarized in Table 15.

In general these results are similar to those reported in Tables 6 - 10 in that there was a rapid rise in the quantity of destabilized fat during normal freezing which progressively increased as the time in the freezer was extended. Three of the eight emulsifiers, Atmos 150, Atmos 300 and Tween 65 caused extensive destabilization. This was particularly evident after the ice creams were subjected to additional agitation in the freezer, but none of this group of emulsifiers caused the extensive destabilization produced by Tween

80, Tables 6 - 10. With few exceptions the destabilization was considerably below that found in the control ice cream. Under all conditions of agitation the ice creams containing Myverol 18-40 caused the least destabilization of the butterfat.

TABLE 15. The effect of an additional number of emulsifiers, emulsifier concentration and varying degrees of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat

			Destabilized Fat (%)			
Emulsifiers (Trade name)	Chemical Composition	Rate of Addition %	Mixes	Normal Freezing Time (Ca. 10 min)	Additional Agitation 15 min	30 min
None (Control)	-	-	7.3	15.9	49.3	59.6
Atmos 150	2/3 mono ad	0.1	8.8	22.4	45.6	56.0
" "	1/3 diglyceride	0.2	8.9	20.8	44.1	60.2
Atmos 300	mono ad	0.1	8.7	20.5	46.7	66.2
" "	diglyceride	0.2	9.5	18.5	41.0	60.2
Myvecet 7-00	acetylated	0.1	6.9	11.5	33.3	44.1
" "	hydrogenated lard	0.2	7.0	11.9	26.3	45.3
Myverol 18-40	40% saturated	0.1	6.7	10.3	26.2	34.6
" "	ad 60% unsaturated	0.2	7.4	15.2	25.1	37.4
Myvetex 8-20	80% Myverol ad	0.1	7.5	15.8	27.1	50.3
" "	20% hydrogenated veg. oil	0.2	8.3	17.3	27.1	40.3
Span 40	Sorbitan	0.1	8.9	14.8	20.7	44.8
" "	monopalmitate	0.2	6.4	15.9	26.4	39.1
Span 80	Sorbitan	0.1	8.5	19.9	28.1	47.6
" "	mono-oleate	0.2	7.8	18.4	29.1	43.3
Tween 65	polyoxyethylene	0.1	8.1	12.9	31.1	48.9
" "	Sorbitan tristearate	0.2	7.8	18.6	46.9	66.2

The Influence of Stabilizing Salts.

Sodium hexametaphosphate (Calgon), sodium oxalate and sodium tetraphosphate were added to 10% fat mixes, made from the same ingredients, before pasteurization at the rate of 0.1 and 0.2%. Two series of mixes were prepared, one containing Span 60 emulsifier and the other Tween 80 emulsifier. These mixes were then subjected to freezing and agitation, and analyzed for destabilized fat. The results are shown in Table 16, and Figs. 17 - 20.

The three sodium salts were effective in reducing the destabilization of the ice creams, but sodium tetraphosphate was particularly effective, especially under extreme agitation and with Tween 80 emulsifier. The most effective concentration of the salt was 0.2%.

TABLE 16. The effect of incorporation of sodium salts and of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat.

Salts Additives	Rate of Addition %	Destabilized Fat (%) Mixes	Normal Freezing Time (Ca. 10 min)	Additional Agitation 15 min	30 min
Span 60 Series					
Control (no emulsifier)	-	7.4	17.2	48.7	52.3
Control - emulsifier	0.1	8.3	12.5	31.4	36.2
Sodium hexametaphosphate	0.1	7.1	13.9	24.4	31.2
" "	0.2	8.4	12.3	23.8	29.5
Sodium oxalate	0.1	8.5	15.3	23.6	28.4
" "	0.2	8.2	11.0	20.2	22.0
Sodium Tetraphosphate	0.1	7.4	10.8	21.9	26.6
" "	0.2	8.6	10.7	19.4	21.8
Tween 80 Series					
Control (no emulsifier)	-	7.4	17.2	48.7	52.3
Control - emulsifier	0.1	7.4	19.0	54.3	75.7
Sodium hexametaphosphate	0.1	8.5	15.7	51.2	68.7
" "	0.2	7.5	15.3	40.4	68.5
Sodium oxalate	0.1	8.4	18.7	46.2	55.5
" "	0.2	8.4	14.8	40.0	45.1
Sodium Tetraphosphate	0.1	8.7	13.4	40.8	50.0
" "	0.2	8.5	12.2	33.3	42.7

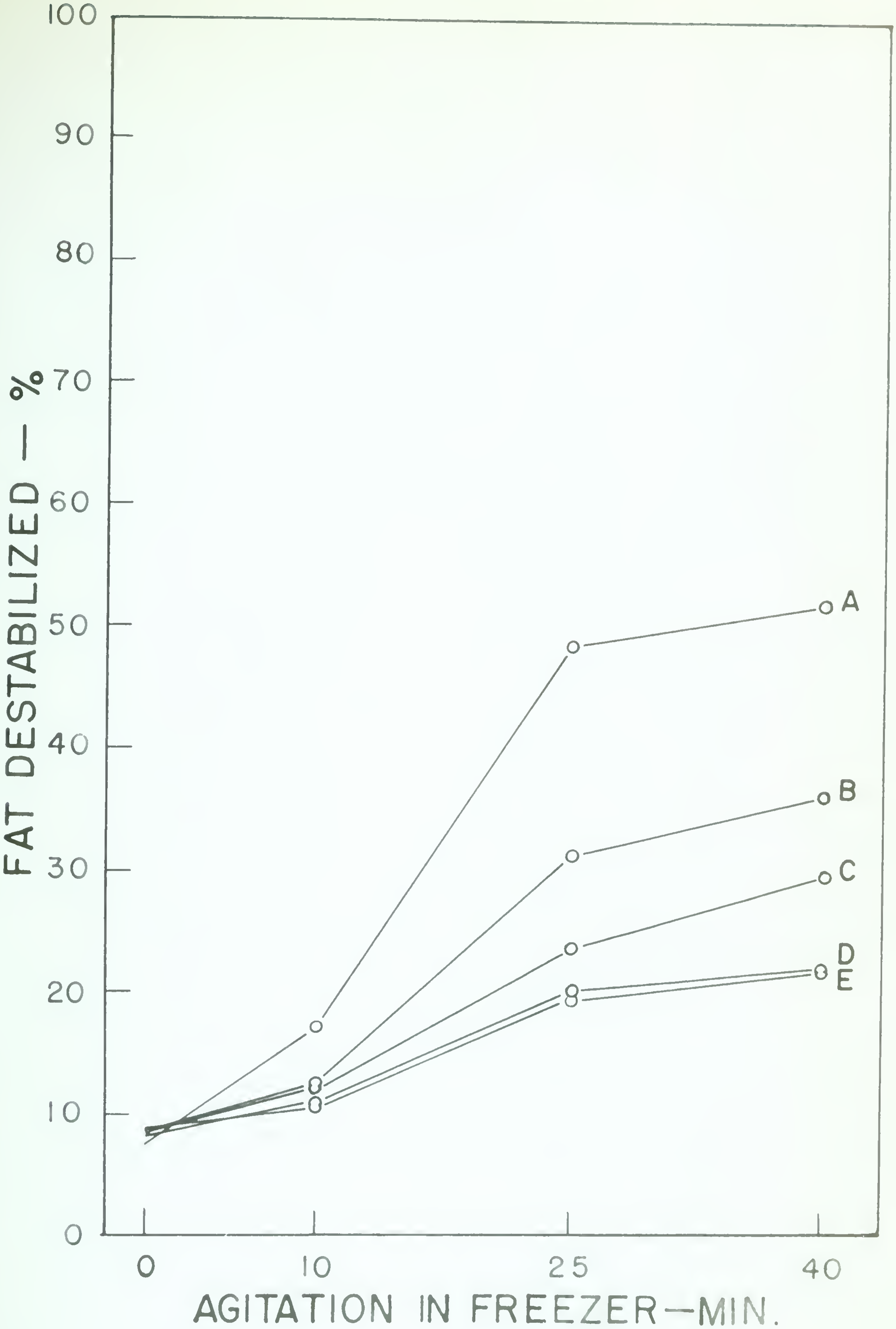

Fig. 17. The effect of incorporation of sodium salts at 0.2% and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat and 0.1% Span 60 emulsifier.
(A) Control (B) Control - Span 60 (C) Sodium Hexametaphosphate (D) Sodium Oxalate (E) Sodium Tetraphosphate

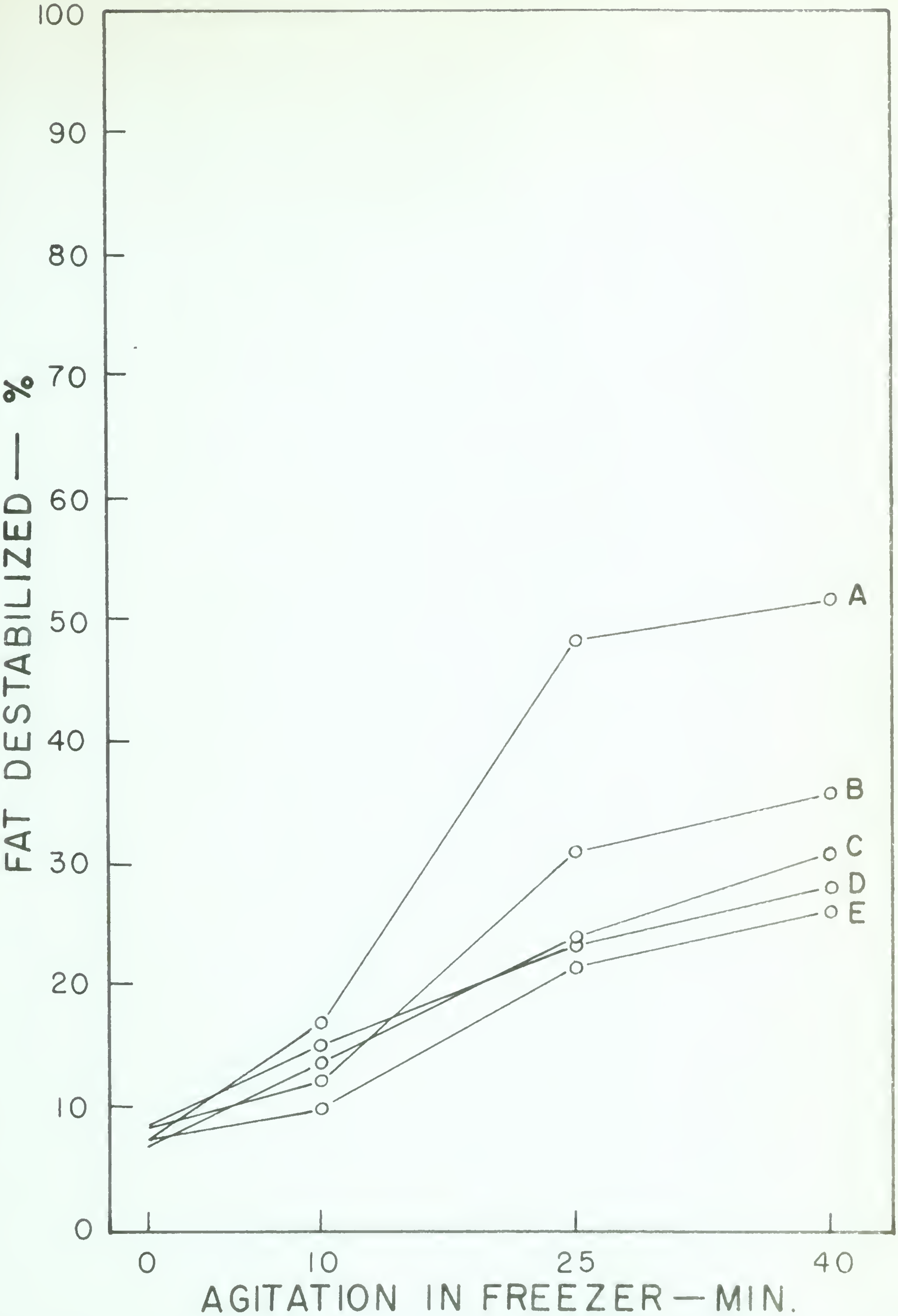

Fig. 18. The effect of incorporation of sodium salts at 0.1% and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat and 0.1% Span 60 emulsifier.
(A) Control (B) Control - Span 60 (C) Sodium Hexametaphosphate (D) Sodium Oxalate (E) Sodium Tetraphosphate

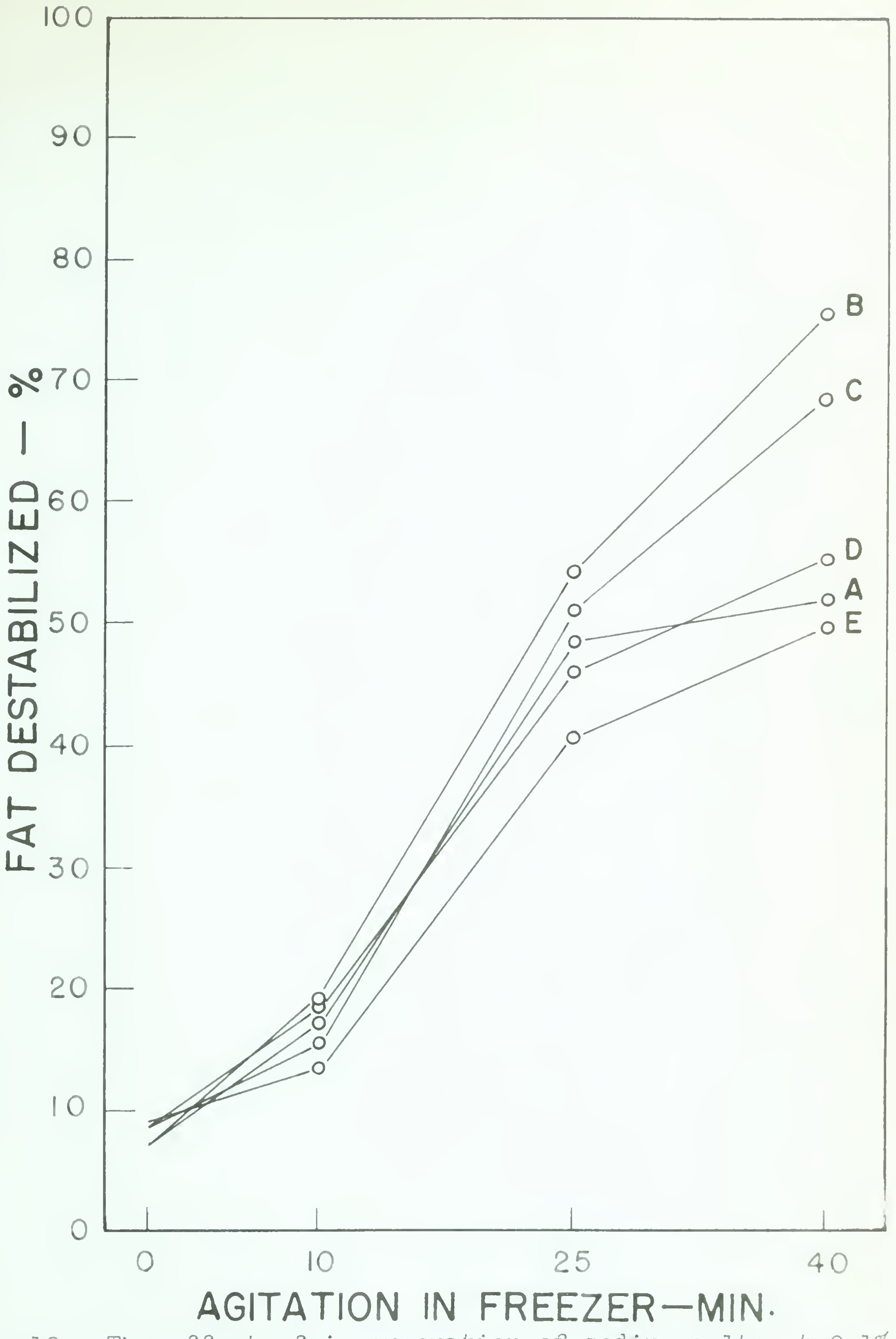

Fig. 19. The effect of incorporation of sodium salts at 0.1% and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat and 0.1% Tween 80 emulsifier. (A) Control (B) Control - Tween 80 (C) Sodium Hexametaphosphate (D) Sodium oxalate (E) Sodium Tetraphosphate

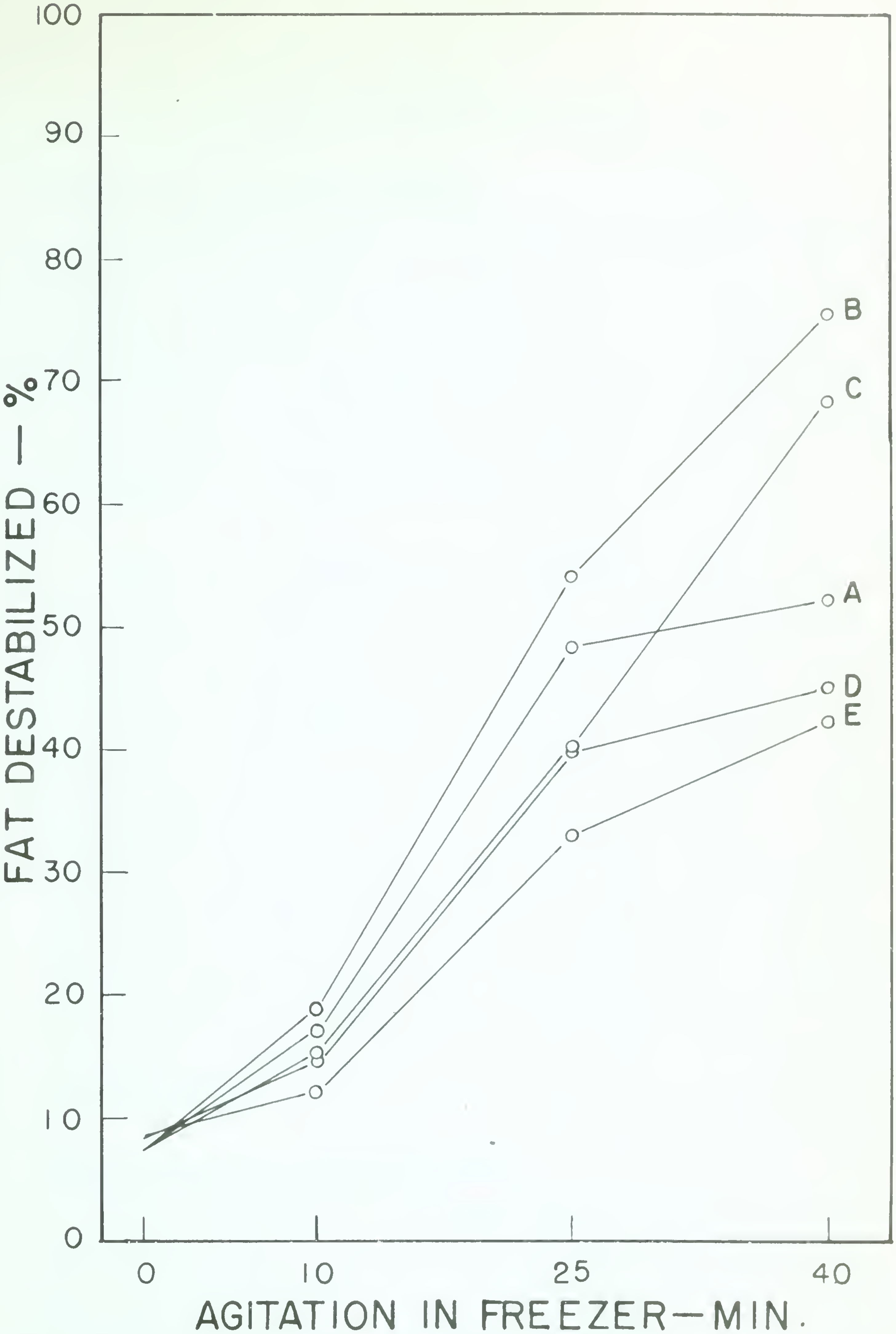

Fig. 20. The effect of incorporation of sodium salts at 0.2% and the degree of agitation on the destabilization of fat in mixes and corresponding ice cream containing 10% butterfat and 0.1% Tween 80 emulsifier.
(A) Control (B) Control-Tween 80 (C) Sodium Hexametaphosphate (D) Sodium Oxalate (E) Sodium Tetraphosphate

Microscopical Examination

Examination of Melted Samples.

The procedure used in examining the melted samples proved to be satisfactory. By this procedure it was possible to detect fat globules and butter granules. The dilution of 1:100 was used in order to reduce the concentration of the material examined, and this assisted in obtaining clearer pictures.

The staining solutions successfully colored the fat globules and destabilized fat. The fat globules appeared yellow, while the destabilized fat appeared as dark patches scattered throughout the solution. By this procedure the effect of different emulsifiers on the stability of fat in ice cream with normal freezing and additional agitation was recognized. The coloring of the fat globules and destabilized fat with neutral red and Sudan III was in agreement with the findings of King (1955b), who used fluorescent light in his investigation.

Examination by Polarized Light

The photomicrographs presented in Plates 1 and 2 illustrate the appearance of the fat globules and destabilized fat in mixes and corresponding ice cream containing different emulsifiers under conditions of

normal freezing and extended agitation. The globular or undestabilized butterfat can be identified by the peripheral birefringency of the enclosed crystalline butterfat that gives the globule the appearance of a luminous tetrad, according to King (1955b). As the butterfat becomes destabilized by freezing, agitation, and the action of some emulsifiers, the non-globular butterfat gathers into agglomerates of incipient butter granules which are easily recognized. The photomicrographs in Plate 1 show that most of the fat in the ice cream mixes is still in the globular form. However, mixes containing Tween 80 emulsifier show more crystalline butterfat covering the surface of the fat globules which obscures them, and they are not so easily recognized as the globules in the Control or Span 60 emulsifier-containing mixes. It can be seen that as the mixes are frozen and exposed to additional agitation the globular butterfat tends to disappear and be replaced by agglomerates and butter granules. This is especially noticeable with ice creams containing Tween 80 emulsifier exposed to 30 minutes additional agitation. Photomicrographs in Plate 2 show the same trend for mixes and corresponding ice creams prepared from butter oil. The appearance of the globular fat in the mixes is not as distinct as those in mixes made from cream, while the presence of agglomerates and butter granules indicates

that the effect of freezing and agitation is more severe on ice cream made from butter oil. Further investigation proved that this procedure can be used to examine mixes and ice cream samples which have been freeze-dried, provided the samples are dissolved in mineral oil.

Electron Microscope Examination.

The electron photomicrographs, Plates 3 and 4, show the appearance of the fat globules and membrane materials in ice creams containing different emulsifiers with normal freezing and additional agitation. With this procedure it was possible to observe the changes that occurred in the fat globules exposed to severe agitation in the freezer. The appearance of small, homogenized fat globules in chain-like structure around the small air cells was recognized. It is interesting to see that the fat globules in the control sample exposed to normal freezing conditions are covered by a layer which seems thicker than that covering the fat globules of samples containing emulsifiers. The membrane material surrounding the fat globules in samples containing Tween 80 emulsifier, Pl. 3 (e) and (f), appears to be thinner and weaker than those containing Span 60 emulsifier, Pl. 3 (c) and (d). The appearance of the stretched

Shape of the fat globules in samples receiving additional agitation indicates that agitation causes stretching of the membrane material to increase the size of the fat globules. The photomicrographs also show that the surface layer covering the fat globules receiving little disturbance is thicker than that surrounding the fat globules which have been exposed to additional agitation. The photomicrograph of the sample containing Tween 80 emulsifier with 30 minutes additional agitation show mostly membrane material.

It was found that artificially produced fat globules have thinner surface layers than those of the natural fat globules. This can be seen by comparing photomicrographs of Plate 3 with those in Plate 4.

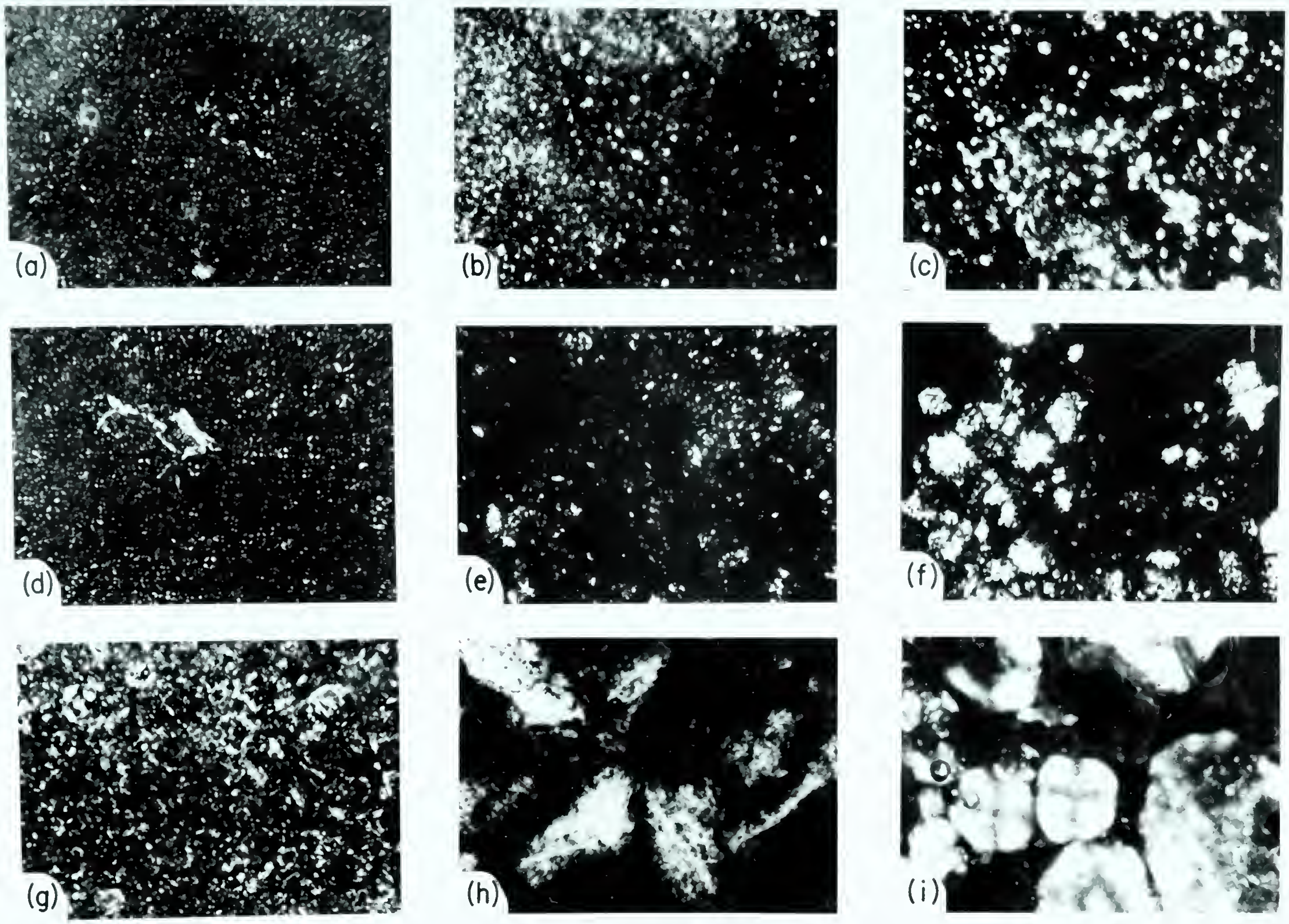

Plate 1. Polarized light photomicrographs of destabilized fat in 10% butterfat mixes and corresponding ice creams. Butterfat source - cream.

(a) Control - mix (b) Control - normal freezing (c) Control - 40 min agitation
(d) Span 60 - mix (e) Span 60 - normal freezing (f) Span 60 - 40 min agitation
(g) Tween 80 - mix (h) Tween 80 - normal freezing (i) Tween 80 - 40 min agitation

Magnification 750x

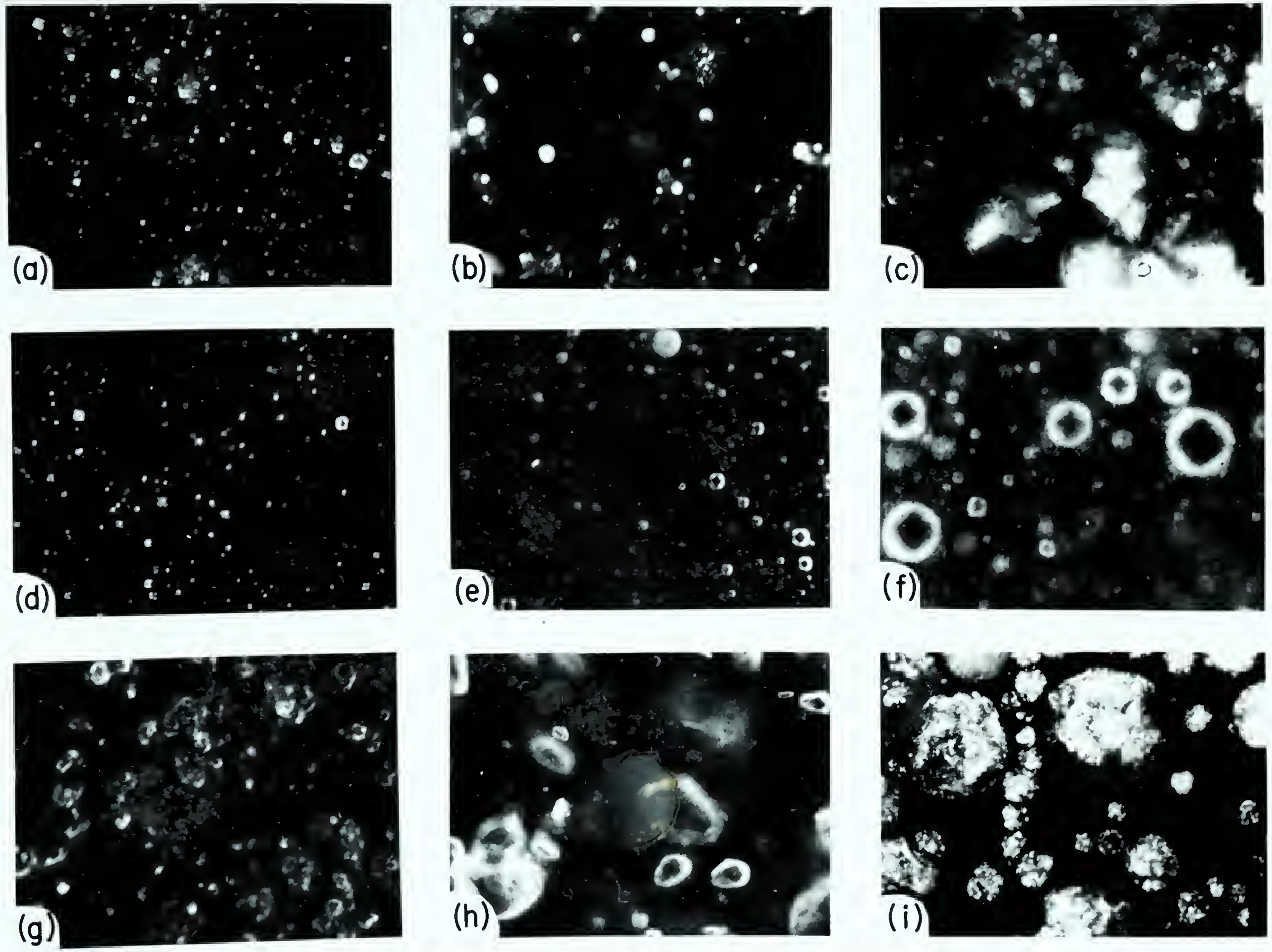

Plate 2. Polarized light photomicrographs of destabilized fat in 10% butterfat mixes and corresponding ice creams. Butterfat source - butter oil.

(a) Control - mix (b) Control - normal freezing (c) Control - 40 min agitation
(d) Span 60 - mix (e) Span 60 - normal freezing (f) Span 60 - 40 min agitation
(g) Tween 80 - mix (h) Tween 80 - normal freezing (i) Tween 80 - 40 min agitation

Magnification 750x

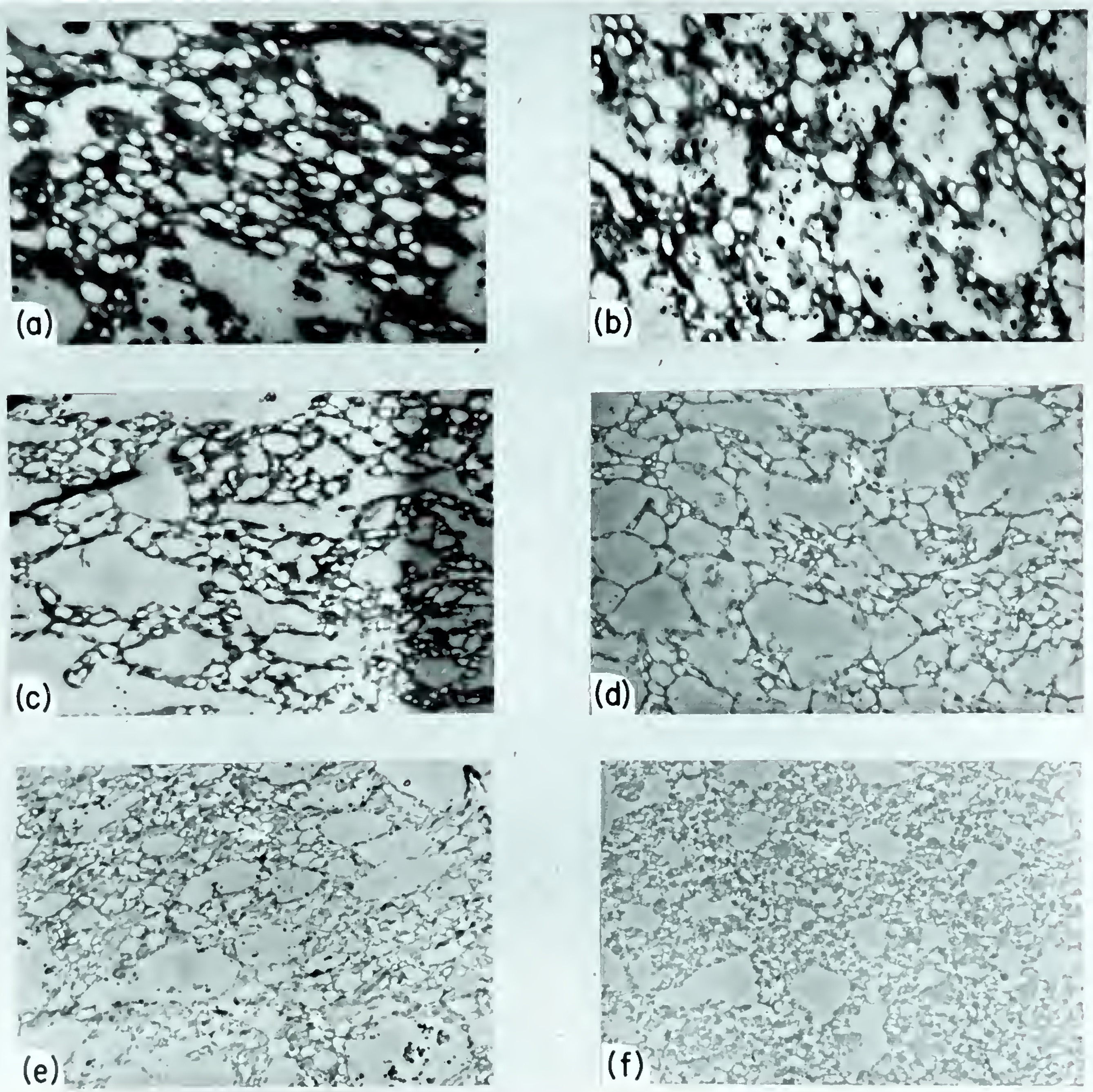

Plate 3. Electron microscope photomicrographs of destabilized fat in 10% butterfat ice creams. Butterfat source - cream.

(a) Control - normal freezing
(b) Control - 40 min agitation
(c) Span 60 - normal freezing
(d) Span 60 - 40 min agitation
(e) Tween 80 - normal freezing
(f) Tween 80 - 40 min agitation

Magnification 30,000x

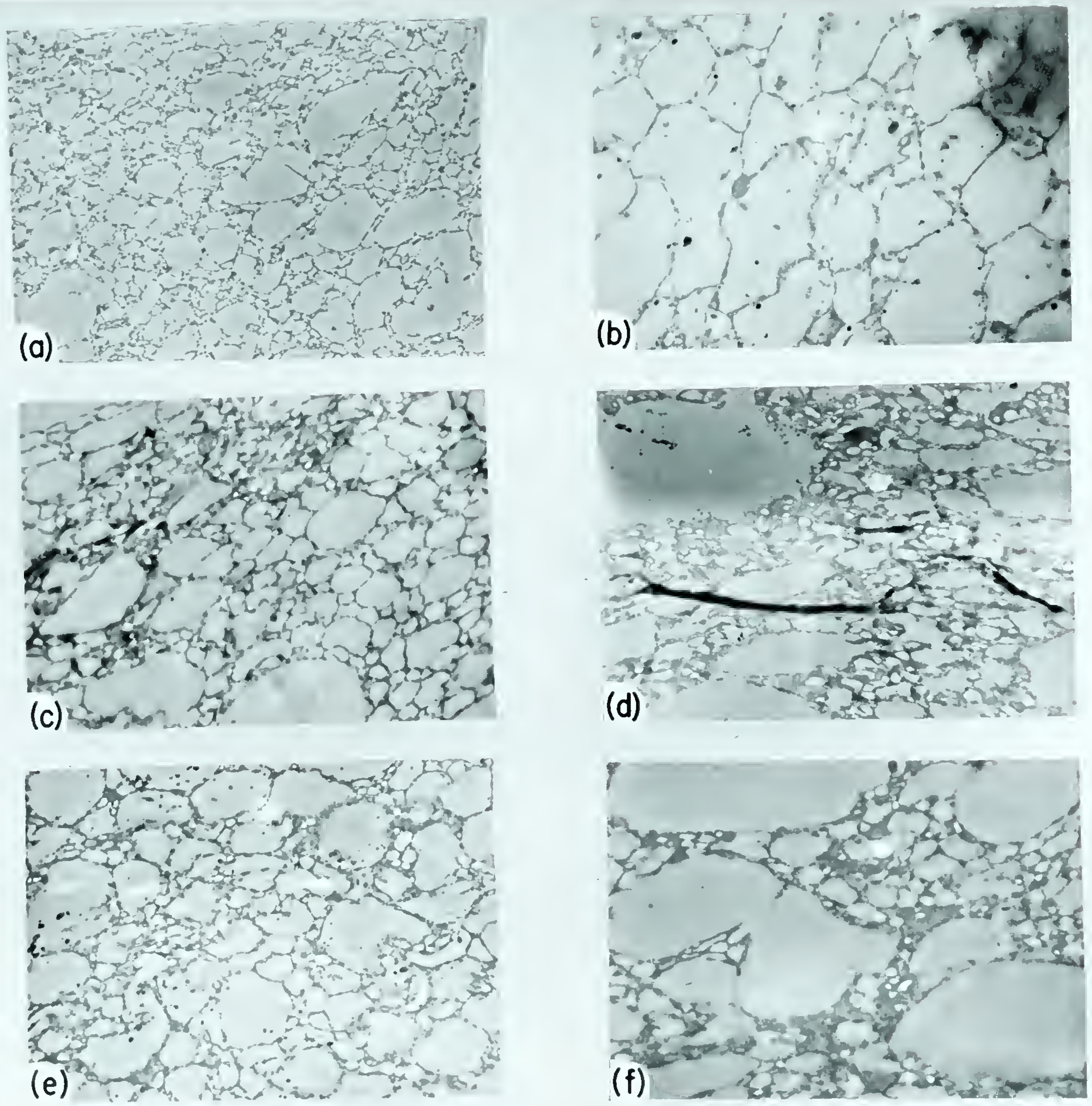

Plate 4. Electron microscope photomicrographs of destabilized fat in 10% butterfat ice cream. Butterfat source - butter oil.

(a) Control - normal freezing
(c) Span 60 - normal freezing
(e) Tween 80 - normal freezing
(b) Control - 40 min agitation
(d) Span 60 - 40 min agitation
(f) Tween 80 - 40 min agitation

Magnification 30,000x

DISCUSSION

The Effect of Fat Content and Source of Butterfat.

In spite of the seriousness of the destabilization of the milk fat emulsion of ice cream, especially in "soft-serve" ice cream which is still frozen by the batch process and subjected to extensive additional agitation after freezing, little research effort has been expended on this problem. Most of the information on the destabilization of the milk fat of ice cream is based on subjective tests such as the appearance or tactile impression of a defective product although ice cream has been examined under the microscope to determine the extent of destabilization (Valear,1960 and Cremers,1954). Only recently have methods for determining the extent of destabilization quantitatively received serious consideration. (Keeney and Josephson, 1958; Keeney and Kloser, 1958; and Keeney, 1962).

Statements frequently appear in the literature indicating that destabilization of the ice cream emulsion increases with increases in the fat content, but no quantitative information is presented. It is generally assumed in the trade that a reduction of the present Canadian minimum standard of 10% butterfat would greatly reduce difficulty experienced with churning of ice

cream during freezing, but little, if any, information is available on the actual effect of such a reduction of the butterfat content or whether the selection of a particular emulsifier or salt additive would be equally or more effective.

The destabilized fat content of the ice creams made in this investigation were surprisingly high and, even under conditions of extended agitation in the freezer exceeded the value of 55% reported by Keeney and Josephson (1958) for the ice cream in certain novelties and the 44% destabilized fat reported by Kloser and Keeney (1958) when a 14% fat mix had been agitated in a freezer for 30 minutes. The results also show a considerable quantity of destabilized fat in the mixes apparently caused by the agitation of the processing procedure which has been overlooked or disregarded by many investigators. The data presented show increases in destabilized fat from 4.5 to 13.4% for 5% butterfat increments of the composition of the ice cream made from cream, (Table 11). Under conditions of additional agitation these increases reached 30.1%. Little information is available in the literature on the destabilization of the butterfat of ice cream made from destabilized butterfat obtained from butter oil

and butter which are re-emulsified in processing an ice cream made from such butterfat sources. Such artificially emulsified mixes might be expected to have an excessive quantity of destabilized fat when frozen into ice cream, especially if the agitation was excessive, as it frequently is in a counter freezer. The results obtained in this investigation do, in general, show a greater percent of destabilized fat, but the quantity present was unexpectedly low. The increases of destabilized fat for each 5% increment in the butterfat content of the ice creams ranged from 4.0 to 14.6% under normal freezing conditions. Under conditions of extended agitation the increases for each increment of 5% butterfat ranged from 6.1 to 24.3% (Table 11). These results provide good evidence that the destabilized fat content of ice cream made from butter oil should not normally differ much from the quantity of such fat present in ice cream made from cream as a source of butterfat.

The Effect of Type and Concentration of Emulsifier.

The results reported throughout this research showed that of the emulsifiers investigated, Tween 80 and the other liquid emulsifiers, and Span 60 caused the greatest and least destabilization respectively. This is in accord with the findings of Kloser and Keeney (1958),

Keeney and Josephson (1958) and Knightly (1959). However, Frazuer (1959c) reported that Tween 80 and Span 60 both reduced churning time below that of the control. The results reported by the latter investigator were based on sensory testing of the churned fat and not on the quantitative determination of the destabilized butterfat.

The chemical and physical reactions of emulsifiers in ice cream and other food products is incompletely understood and, consequently, there is a paucity of published information on this aspect of ice cream making. This leads to speculation regarding the action of emulsifiers in promoting or retarding destabilization of the butterfat emulsion. Some recent investigations by King (1957), Bassett (1958), and Mather and Hollender (1955) shed some light on this problem. According to Bassett, the "poly's" type of emulsifier, such as Tween 80 (a polyoxyethylene derivative of sorbitan), orientates its molecules loosely on the surface of the fat globules, and under conditions where there is a continuous agitation at low temperature, they act as foam dispersers. It is a known fact that a fat emulsion is more stable when the air system is stable. King (1957) observed that

liquid Tween 80 and Span 80 both exhibit a sweeping effect of the fat globules and clumps from the surface of the milk. This undoubtedly was caused by increasing the hydrophobic property of the surface layer of the fat globules. Mather and Hollender (1955) found that Tween 80 emulsifier, when added to whole milk powder, caused the product to become self-dispersing, but churning occurred on reconstitution with mechanical agitation. They attributed the churning effect to the improper balance of the hydrophilic - lipophilic properties of the emulsifier. The churning property disappeared when a more favorable balance between the hydrophilic and the lipophilic properties was reached by using another emulsifier which was lipophilic. Based on these findings, it seems logical to speculate that the improper balance between the hydrophilic and lipophilic properties of Tween 80 is the cause of a high fat destabilizing effect of this emulsifier in the present research.

According to Pyenson and Dahle (1938), the phospholipid portion of the membrane material of fat globules is the most hydrophilic component of any substance isolated from milk. Since Tween 80 and other liquid emulsifiers are also strongly hydrophilic, it is possible that this type of emulsifier disturbs

the adsorbed surface layer and creates more contact between the phospholipid portion and the emulsifier. If this occurs, the surface layer becomes weaker and butterfat globules show less resistance to churning. Smith and Jack (1959) reported that the fatty acid components of the phospholipid material is mostly unsaturated. Since Tween 80 contains mono-oleate, an affinity probably exists between the unsaturated fatty acids of the phospholipid and those of the emulsifier causing an increase in the hydrophobic property of the surface of the fat globules. An increase in hydrophobization of the fat globule surface is the first stage in demulsification, according to King (1955a). It is interesting to note that the emulsifiers investigated in the present research which contained long-chain saturated fatty acids caused less fat destabilization than those with long-chain unsaturated fatty acids of mono- and diglycerides.

Increases in the concentration of the emulsifiers under investigation showed an increase in the percent of destabilized butterfat. This increase is more noticeable after additional agitation, especially with Tween 80. This is probably caused by over-emulsification. The over-emulsification condition may be created by

further deviation from the proper balance of the hydrophilic-lipophilic properties of the emulsifiers. It is also possible that an increase in the concentration of Tween 80 and other liquid emulsifiers caused an increase in the polyglycol chain, which effects the fat emulsion by its chemical and physical reactions.

The Effect of Stabilizing Salts.

The ice creams containing sodium oxalate, sodium hexametaphosphate and sodium tetraphosphate showed less butterfat destabilization than the controls. The decrease was approximately 10% and 20% for the Span 60 and Tween 80 series respectively (Table 16). It was found that an increase in the concentration of the salts from 0.1 to 0.2% showed a marked decrease in the quantity of destabilized fat, especially in the samples containing Tween 80 emulsifiers. Sodium oxalate is not a salt that could be used in ice cream because of its toxicity. It was only included in the study because of its calcium ion sequestering property. Of the three salts added, sodium tetraphosphate at 0.2% showed the greatest effect in reducing destabilization. This confirms the work of Keeney (1962). The action of these salts on the stability of the fat emulsion in ice cream is not completely

understood, and a complete answer must await further investigations into the physical chemistry of ice cream. Any explanation given is based on speculation. Doan (1930), Keith *et al*. (1935), and Dahlberg and Hening (1929) studied the effect of salt balance on the stability of protein in ice cream. In general, they found that the use of sodium citrate, carbonate and phosphate increased protein stability and reduced fat clumping. This might be attributed to the stabilizing effect of these salts on the protein portion of the adsorbed surface layer of the fat globules. Dahle and Rivers (1940) investigated the effect of sodium hydroxide, calcium hydroxide and magnesium oxide on the stability of the protein in ice cream. They concluded that the stability contributed by these chemicals to the proteins of the ice cream is probably related to the increase in the pH brought about by the salts. When the pH is increased, the proteins are removed from their isoelectric point and their stability is increased. Frazeur (1958a) reported that sodium citrate, disodium phosphate, tetrasodium pyrophosphate and sodium hexametaphosphate, when added to 10.5% butterfat ice cream before pasteurization at 0.1%, lengthened the churning time. He concluded that these salts, when added to ice cream, increase the electrostatic charges, causing them to repel

each other. Knightly (1959) stated that calcium salts usually cause emulsion destabilization, while citrate and phosphate show a stabilizing effect on the fat emulsion in ice cream. Keeney (1962) speculated that the stability brought about by citrates and phosphates involves a mechanism which affects the stability of the protein. He did not state whether the protein complex of the fat globules or other milk protein is involved. He also speculated that these salts may add certain strength to the lamellae of the air cells. Based on the information available and the results reported in this research, it may be logical to speculate that the salts added are adsorbed on the surface of the fat globules causing them to repel each other by decreasing the hydrophobizing property of their surfaces. This may be caused by making the surface of the fat globules less attracted to the free fat. It is also possible that the presence of the ions of these salts in combination with some protein from the serum, form a film around the free fat, keeping it from being adsorbed on the surface of the fat globules. If such a film is formed, it will assist in adding some strength to the lamella of the air cells and at the same time, it may act as a foam stabilizer.

Microscopical Examination

Examination of Melted Samples.

Examination of mixes and melted samples of ice cream clearly indicates the effect of freezing, agitation and type of emulsifier on the stability of the fat emulsion in ice cream. This was recognized by observing fat globules and small clumps in mixes and in ice cream samples which did not receive additional agitation in the freezer. On the other hand, agglomerates, incipient butter granules and lumps of fat were observed in samples receiving additional agitation, especially those containing Tween 80 emulsifiers. This finding confirms the finding reported by Valear (1960).

The successful coloring of the fat globules and destabilized fat with Sudan III and neutral red is in agreement with the findings of King (1955b), (1959), who used the fluorescent light in his investigation.

Examination by Polarized Light.

The polarized light examination of mixes and corresponding ice creams indicated that it is a satisfactory method for detecting fat globules and destabilized fat. This can be seen from the photomicrographs presented in Plates 1 and 2. The

undestabilized fat was identified by the peripheral birefringency of the enclosed crystalline butterfat that gives the globules the appearance of a luminous tetrad. This confirms the findings of King (1950b). It was found that the luminous tetrad appearance tends to disappear with increase in agitation in the freezer, and it is replaced by some agglomerates and butter granules, depending on the extent of agitation and on the type of emulsifier present. The appearance of the luminous tetrad is clearer in mixes prepared from cream than in mixes prepared from butter oil. This can be seen by comparing photomicrographs in Plate 1 with those in Plate 2. This is probably caused by a difference in the surface layer of the natural fat globules and the artificial ones.

Most of the polarized light examinations of ice cream reported in the literature are related to the study of the ice crystals and air cells, Keller *et al*. (1943), and Blanton (1953).

Electron Microscope Examination.

A review of the literature revealed that the electron microscope has never been used in examining ice cream. However, the fat globules in milk and cream have been examined by the electron microscope. Since this is the first attempt, it needsfurther

investigation and it can be developed into a very useful means of investigating certain characteristics of ice cream structure. In spite of being the first attempt, a very satisfactory result was obtained. It was possible to detect small homogenized fat globules arranged in chain-like structure around the small air cells, especially in samples which received minimum agitation. This confirms the findings of Cremers (1954) who used ordinary light microscope in his examination. It is interesting to find that the fat globules in ice cream containing no emulsifier are surrounded by a surface layer which appears thicker and darker than the surface layer surrounding fat globules in ice cream containing emulsifiers. This may indicate that the emulsifiers influence the adsorbed surface layer of the fat globules. The influence of the emulsifiers on the adsorbed surface layer is not easy to explain. Probably, the emulsifier particles are adsorbed in the areas where it is not completely filled with the adsorbed particles causing the surface to be smoother. It is also possible that the emulsifier removes most of the adsorbed particles causing the surface layer to appear smoother and thinner.

The difference in the appearance of the membrane

surface layer of the undisturbed fat globules and those exposed to additional agitation is easily recognized in the photomicrographs presented in Plates 3 and 4. The surface layer of the agitated ice cream samples appear to be weak and thin, especially those containing Tween 80 emulsifier. Knoop et al. (1959) found that fat globules receiving little disturbance show thicker surface layer than those layers surrounding the disturbed fat globules.

It was also observed that artificially produced fat globules had thinner surface layers than the natural fat globules. This can be seen by comparing photomicrographs in Plate 3 with those in Plate 4. This also confirms the findings of Knoop et al. (1959). Knoop et al. (1958) stated that preparation of the specimen causes some destruction to the membrane material of the fat globules.

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APPENDIX

TABLE 1. Analysis of variance of butterfat destabilization as affected by fat content, different emulsifiers, normal freezing and additional agitation in the freezer. (Butterfat source - cream).

Sources	D.F.	F. Ratio
Replicates	2	4.14
Emulsifiers	3	4.36
Error (1)	6	
Sub-total	11	
Mixes	4	7.70 **
Mixes x Emulsif.	12	2.68 *
Error (2)	32	
Sub-total	59	
Treatments	2	416.90 **
Treatment x Emuls.	6	71.80 **
Treatment x Mixes	8	2.70 *
Treatment x Mixes x Emuls.	24	29.50 **
Error (3)	80	
Total	179	

* Significant at $P = .05$

** Significant at $P = .01$

TABLE 2. Analysis of variance of butterfat destabilization as affected by fat contents, different emulsifiers, normal freezing and additional agitation in the freezer. (Butterfat source - butter oil).

Sources	D.F.	F. Ratio
Replicates	2	4.87
Emulsifiers	3	33.67 **
Error (1)	6	
Sub-total	11	
Mixes	4	7.74 **
Mixes x Emulsif.	12	3.68 **
Error (2)	32	
Sub-total	59	
Treatments	2	722.70 **
Treatments x Emuls.	6	88.90 **
Treatment x Mixes	8	14.40 **
Treatment x Mixes x Emulsif.	24	66.20 **
Error (3)	80	
Total	179	

* Significant at P = .05

** Significant at P = .01

TABLE 3. Analysis of variance of butterfat destabilization in 10% butterfat mixes and corresponding ice creams as affected by concentrations of Tween 80, Span 60 and Myverol 18-00, normal freezing, and additional agitation in the freezer.

Sources	D.F.	F. Ratio
Replicates	2	1.65
Concentration	2	0.24
Emulsifiers	3	15.76 **
Treatment	2	29.91 **
Concentr. x Emuls.	6	0.33
Concentr. x Treatm.	4	0.04
Emuls. x Treatm.	6	3.07 **
Concentr. x Emuls. x Treatm.	12	3.98 **
Error	106	
Total	145	

* Significant at P $\simeq$ .05

** Significant at P $\simeq$ .01